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ANOVELRAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LOBEGLITAZONE BY DESIGN OF EXPERIMENT

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ABSTRACT:
Background: The study aims to integrate theExperiment Planning (DOE)methodologies with High-Performance Liquid Chromatography in the Reverse Phase (RP-HPLC) for the development of an analytical method to estimate lobeglitazone.
Method:
A DOE strategy was applied to maximize critical method parameters for RP-HPLC, including mobile phase composition, flow rate, and column temperature. The method development was carried out using an analytical column Inspire with a C18 (150 × 4.6 mm,5 µm).stationary phase and acetonitrile-containing mobile phase and potassium dihydrogen phosphatethe procedure showed exceptional linearity range at wavelength of 240nm with a correlation coefficient (R^2) of 0.999. The method was validated according to ICH guidelines, showing satisfactory results in terms of specificity, sensitivity, repeatability, and robustness.
Results: The enhanced RP-HPLC technique demonstrated high precision and accuracy in the estimation of lobeglitazone. The DOE approach enabled systematic optimization, resulting in a robust method with improved sensitivity and reduced analysis time.
Conclusion: The integration of with DOE in RP-HPLC method development offers a strategic approach to enhance analytical method performance. This study successfully established a reliable and efficient RP-HPLC technique for determining lobeglitazone, which can be potentially applied in quality control and pharmacokinetic studies.
Keywords: lobeglitazone, Design of Experiments, RP-HPLC, Method Development, Anti diabetic Drug.

INTRODUCTION:

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disease characterized by insulin resistance and β -cell dysfunction.[1] because the Pathophysiology of T2DM is complex and multifactorial, a variety of oral anti -diabetic agents have developed based on the underlying mechanisms associated with T2DMOne of the classes of OADS that has been developed thus far is the thiazolidines class [2]. TZDSs increase insulin sensitivity by activating the peroxisome proliferator-activated receptor (PPAR). Due to hepatotoxicity, the primary TZD approved for TZDM, troglitazone, was taken off the market in 2000. [3] Pioglitazone and rosiglitazone are two additional TZDS that have been approved by the United States. Due to their potent glycemic durability, they have been widely used to treat type 2 diabetes since being approved by the Food and Drug Administration in 1999.Lobeglitazone was at first developed by chon Kun Darn Drugs in Korea to treat diabetes on July 4, 2.13, the service of food and medication wellbeing (Korea) supported by lobeglitazone, i.e., the Drugs under brand name Duvie. [4] Duvie is a tablet that is taken orally and contains 0.5 milligrams of free lobeglitazone. As directed, take 0.5 mg once every five days. Due to India's high rate of insulin resistance, Glenmark Pharmaceuticals Limited was the first company to market lobeglitazone as a treatment for type 2 diabetes there lobeglitazone (0.5 mg) is present in the medication, which is sold under the brand name LOBG and must be taken orally by patients once daily.[6],[7] It is prescribed to patients with insulin resistance to improve glycemic control. According to the FDA reports, Glenmark recently got endorsement from drugs controller, the medication control directorate general of India(DCGI), to make and market [8] [9]lobeglitazone in view of randomized twofold - tie preliminary in stage 3 patients/individual matured 18yrs, and over who had type 2 diabetes.Insulin obstruction is an engineered made by your pancreas that behaves like a key to allow blood to sugar in to the cells in to your body for use energy. On the off chance that you have type 2 diabetes cells don't answer regularly to insulin. The term for this is insulin resistance. High glucose levels are bad for the body and can lead to kidney disease, heart disease, vision problems, and other serious health issues.

SYMPTOMS: Increases thirst, frequent urination, increased hunger, Unintended Weight loss, Blurred Vision, Fatigue

DRUG PROFILE:

Lobeglitazone is an antidiabetic medication from the thiazolidine class of drugs. it essentially capability as an insulin sensitizer by proliferator by restricting and enacting peroxisome proliferator-initiated Receptors (PPAR) gamma inside fat cells. [10] By enacting PPAR-gamma and advancing the limiting of insulin at fat cells, [11]lobeglitazone accordingly has been displayed to decrease glucose levels, lower hemoglobin A1C (HB1C) levels and further develop lipid and liver profiles.dissimilarto pioglitazone, a double drug.PPAR agonist at PPAR - alpha and PPAR - gamma, Lobeglitazone is an unadulterated Standard alpha agonist. Lobeglitazone was created by presenting a P-methoxy phenoxy bunch at the fourth place of pyramid bunch in the structure of rosiglitazone. Lobeglitazone has a higher binding affinity than pioglitazone and rosiglitazone [12, 13]

Lobeglitazone has well -tolerated anti -diabetic therapy in healthy females.Lobeglitazone is a novel thiazolidineagent with similar glycemic efficacy that reduces insulin resistance. Lobeglitazone decreased the gamble of cardiovascular complications. [14]Lobeglitazone was endorsed by providing a Food Service and Wellbeing (South Korea) in 2013, and is being checked by post advertising observation until 2019. Lobeglitazone isn't supported for use by

either the Food or Medication Organization (USA) Wellbeing Canada, or European Drugs Office Enemy use in the administration of diabetes.

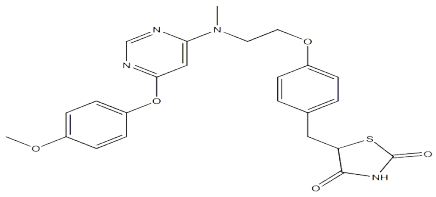


Figure no: 1. structure of Lobeglitazone[14]

This research presents a novel approach to estimate lobeglitazone using a design of experiment (DOE). RP-HPLCmethod. Design of Experimental is a method of statistical analysis that allowsresearchers to optimize experimental parameters systematically, leading to more efficient analytical method. The pharmacophore lobeglitazone contains 2,4-thiazolidinedione.bunch with an ethyl-benzyl N-methyl amino gathering bound to this as an interfacing join. Its chemical name is5-[4-(2-[6-(-4-Methoxy-phenoxy)-pyrimidine-4-y1-methyl-amino-ethoxy)-benzyl] thiazolidine-2,4-dione hydrosulphuric acid, C24H24N4O5S is its structural formula. The co-crystal structure of lobeglitazone and PPAR A P-methoxy phenoxy group was added to the fourth position of the pyrimidine moiety in the structure of lobeglitazone[17][18]. The writing survey revealed that a few practical methods were represented by lobeglitazone in RP-HPLC alone and in combination. This study discusses simultaneous estimation of lobeglitazone.

1.To use the DOE approach to systematically assess and optimize important variables the impact the HPLC analysis, such as the injection volume, temperature, column, flow rate, and composition of mobile phase.

2.Creating an RP-HPLC technique with increased sensitivity and accuracy for lobeglitazone based on DOE.

3.Lobeglitazone analytical method of estimation will be developed by RP-HPLC method by optimizing the chromatographic conditions.

4.The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1)

5.The significance of this research lies in its potential to provide a more sensitive and efficient HPLC method for the estimation lobeglitazone thus contributing to the quality control of this drug. Additionally, the utilization of design of experiment principals ensures a systematic and thorough exploration of experimental space.

Experimental Method:

METHOD DEVELOPMENT BY RP- HPLC

Chemicals and reagents:

Lobeglitazone, Water HPLC grade, Acetonitrile, potassium dihydrogen phosphate, Methanol.

METHOD DEVELOPMENT BY RP- HPLC

HPLC system (water alliance liquid chromatography) model 2695 with PDA detector. Inspire C18 (150 × 4.6 m, 5 µm)was used. The mobile phase was composed of potassium dihydrogen phosphate and acetonitrile the various ratios with flow rate of 1 ml/min. HPLC system was operated at ambient temperature

Method optimization: Initially, different pH combinations and quantities of methanol, ammonium acetate buffer, and methanol, phosphate buffer were explored as the mobile phase.

Lastly, the mobile phase was adjusted so as to Acetonitrile with Potassium dihydrogen phosphate (pH 3.0), in proportion 70: 30 v/v respectively.Lobeglitazone was detected by scanning in the 200–400 nm region in methanol diluents from the chosen wavelength of 240 nm in the UV spectrum. This wavelength exhibits good absorption for both medications. The dose form of lobeglitazone was estimated using the established HPLC method.

Preparation of buffer and mobile phase:

Preparation of potassium dihydrogen phosphate buffer: Weigh 6.8 grams of potassium dihydrogen phosphate and transfer it to a 1000 ml container. The volume was also obtained. The potassium dihydrogen phosphate was broken down and weakened by OPA and layer filtration with 0.45 µm.

Preparation of standard solution: Weigh an accurately amount of 50 mg of lobeglitazone reference standard was taken in a 50 ml cleaned and dried volumetric flask this was then diluted with 50ml of diluent. From this stock 1 ml of solution is withdrawn and dissolved in 10 ml of Methanol it is used as working standard solution.

Preparation of Lobeglitazone sample solution: Take 50 mg of the sample, dissolve it in methanol, make 50 ml of it, ultrasonicate it for 20 minutes, and filter it through microfiltration (Stock – A) One milliliter of this stock-A solution is taken out and dissolved in ten milliliters of methanol to create the working solution. After that, a 0.44-micron Injection filter is used to filter it. (Stock solution of the sample of lobeglitazone)

Design of Experiment[19]The medication test of Lobeglitazone was exposed to the plan of analysis process. The Box-Behnken response surface design was used to determine the fundamental facts of factors' effects and interactions on selected method responses. There were 17 runs altogether..

Statistical analysis:

❖ By using ANOVA, the statistical calculations were processed for variables screening and optimization of the method the statistical tools provide the numerical verification of variables and its effect on responses.

Method operable design region: The space known as Design space is created by the many combinations and reciprocities of input elements**.** The Sigma Tech software's contour graphs were used to establish the design space.

Method Verification: The software suggested the optimal method circumstances to accomplish the intended method objectives. The method was verified to check the predictability of the proposed model.

METHOD VALIDATION: [20-22]

1. Linearity:

From the above-mentioned standard stock solution, 0.1, 0.2, 0.3, 0.4, and 0.5 milliliters were pipette into a five 10-milliliter volumetric flask with diluent to produce concentrated solutions of lobeglitazone ranging from 1 to 10 mg/ml. These solutions were then filtered, injected into an HPLC system, and the peak area was measured. positioned a peak area graph between and concentration on the graph. Regression analysis was used to calculate the correlation coefficient.

2. Precision:

Six volumetric flasks each containing 10 milliliters of a diluent were filled with an aliquot of 0.6 milliliters of the standard stock solution. After that, it was filtered, and six replicates were injected into the HPLC system. The area of each injection was measured.

3. Accuracy:

Standard stock solution preparation: a 1000-g/ml standard stock solution was prepared. The Standard stock solution was further diluted with diluent up to the mark by pipetting out 0.6 milliliters into a 10-milliliter volumetric flask.

Preparation of sample solution:

Accuracy solutions at 50% level: Sample 5 mg is weighed, and then transferred to a 10 ml volumetric flask. About Diluent of 7 ml is added the sample is sonicated to dissolve it completely and bring the volume up to the required level. The sample was then injected into an HPLC injector after 0.6 ml of the aforesaid stock solution was pipette out into a 10 ml volumetric flask and the volume was adjusted with diluents.

Accuracy solutions at 100% level: Sample 10 mg is weighed, then transferred to a 10 ml volumetric flask. 2 ml of diluent is added, and the sample is sonicated to dissolve it completely and bring the volume up to the required level. 0.6 ml of the aforesaid stock solution was pipetted out again into a 10 ml volumetric flask, the volume was adjusted with diluent, and the sample was then injected into an HPLC injector.

Accuracy solutions at 150% level: After measuring and transferring 15 mg of the sample into a 10 ml volumetric flask, 2 ml of diluent is added, and the sample is sonicated to dissolve it entirely and bring the volume up to the required level. Subsequent to adding diluent to bring the volume sufficient, pipette out an extra 0.6 ml of the previously mentioned stock arrangement into a 10 ml volumetric flagon and infuse the example into the HPLC injector.

LOD and LOQ:

The limit of detection and limit of quantification was calculated based on the standard deviation of the response and slope of calibration curve.

Results and discussion:

Table no: 1 Box – Behnken design experimental runs

Surface Graph: Retention time

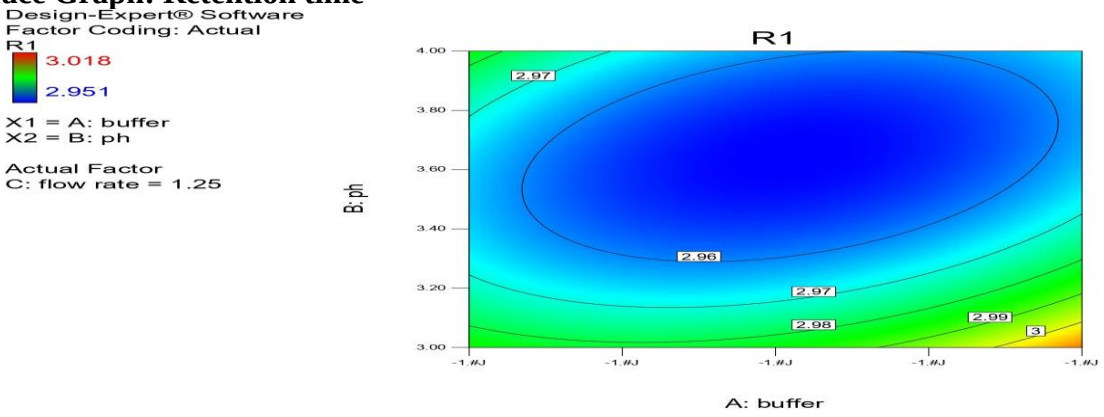


Figure no :2 Retention time

Surface curve Graph: Theoretical plates

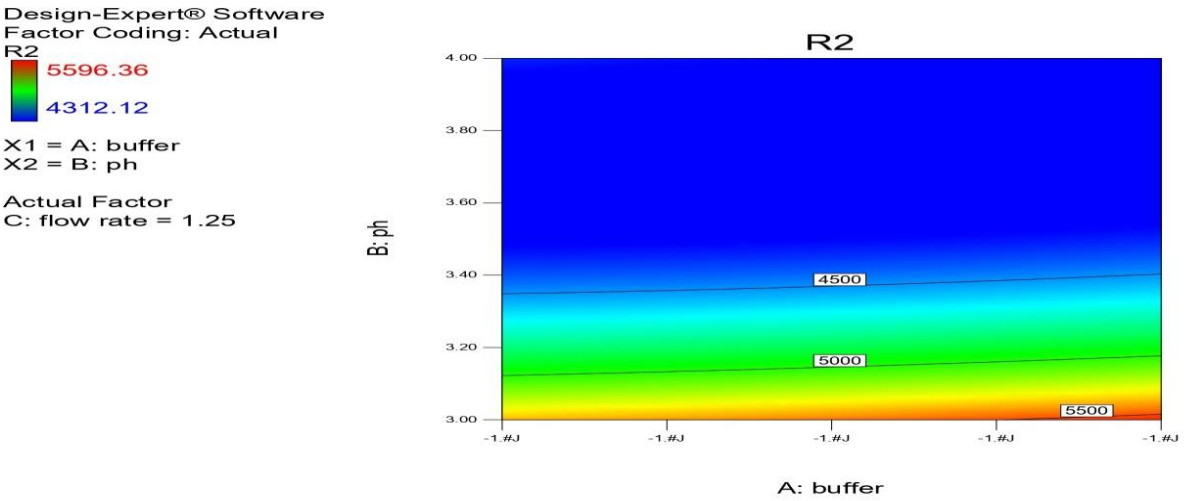
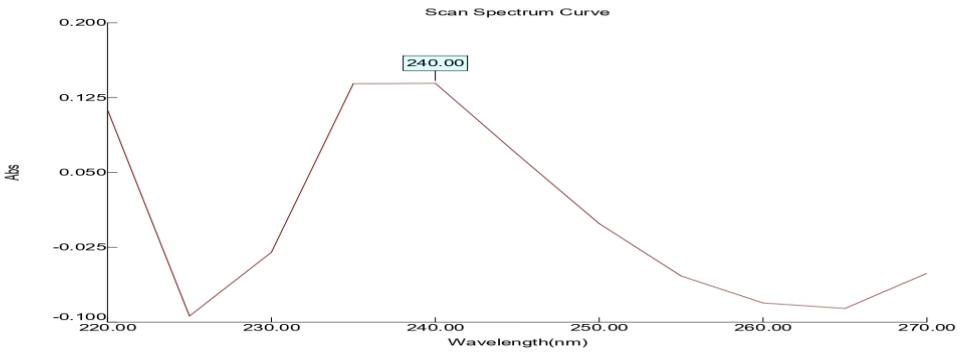


Figure no: 3 - Theoretical plates

Method development

Determination of detection wavelength in mobile phase:



- **Instrument Performance**
  Model : UV-VIS Spectrophotometer
  Number : 30-1885-01-0091
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 220.00 to 270.00 nm
  Measure Mode : Abs
  Interval : 5.00 nm
  Speed : Medium
  Data File : Untitled1.spd
  Create Date/Time : 25 April 2024 14:32:09
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name : Blank
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**
  1 Peak 270.00 -0.051
  2 Peak 240.00 0.139

Figure no:4 Wavelength of lobeglitazone

Validation parameters

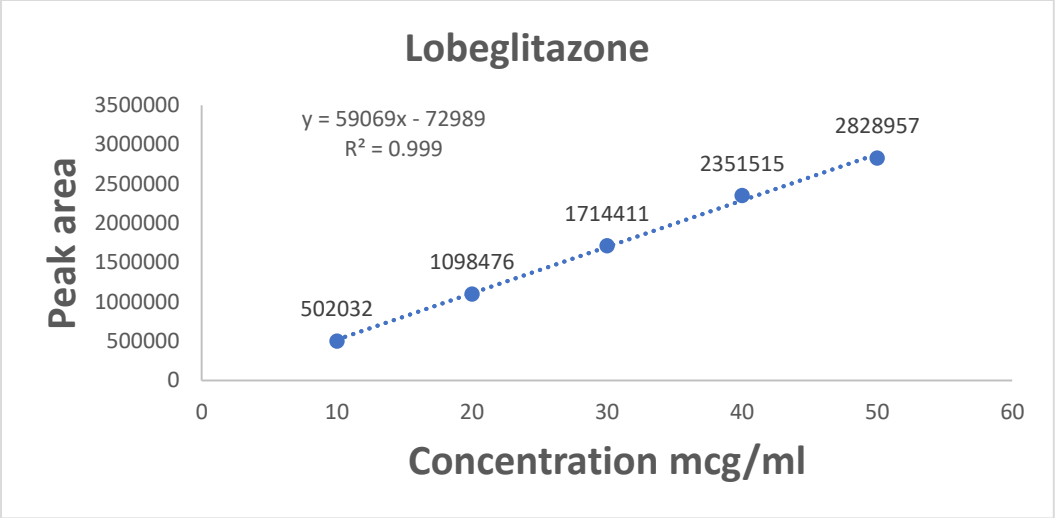
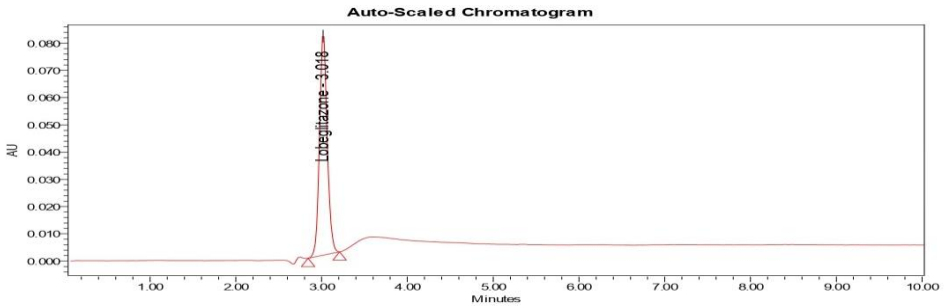


Figure no: 5 Linearity graph of lobeglitazone

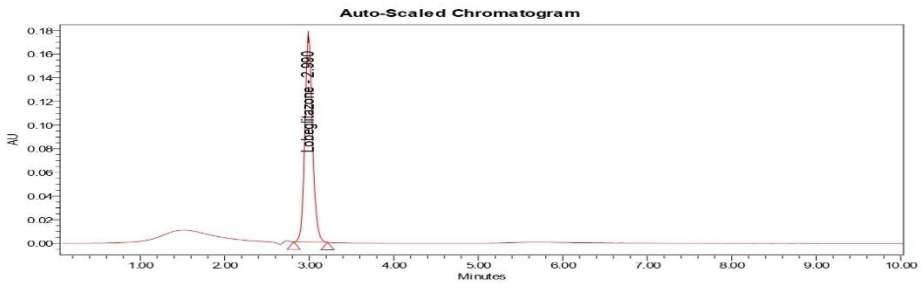
Table no: 2 linearity concentration data

S. No	Linearity Level	Concentration	Area
1	I	10	502032
2	II	20	1098476
3	III	30	1714411
4	IV	40	2351515
5	V	50	2828957
Correlation Coefficient			**0.999**

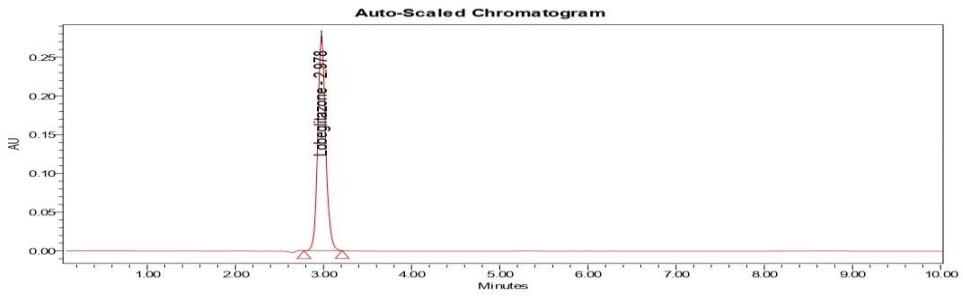
Linearity: 1



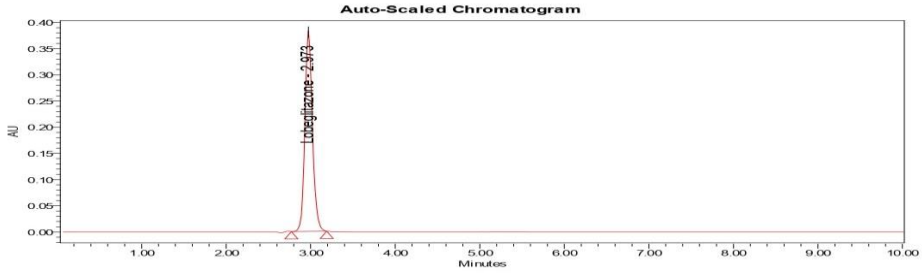
Linearity :2



Linearity:3



Linearity: 4



Linearity: 5

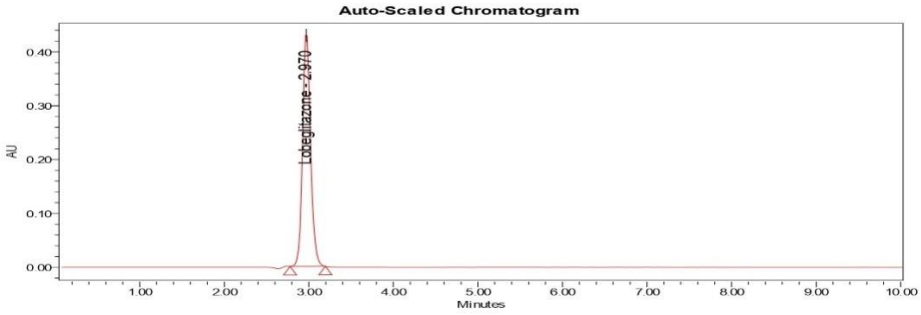
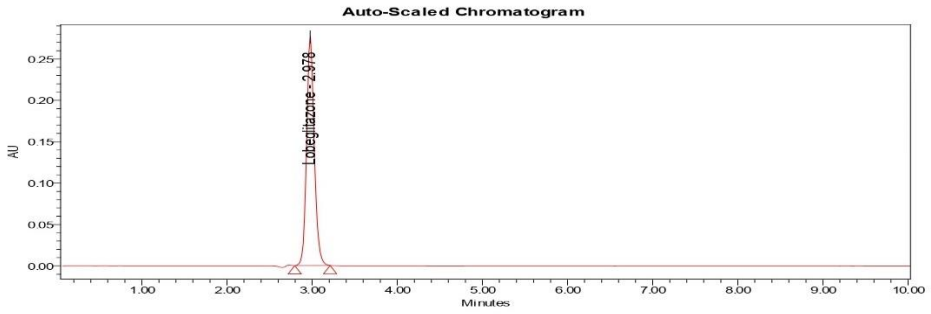
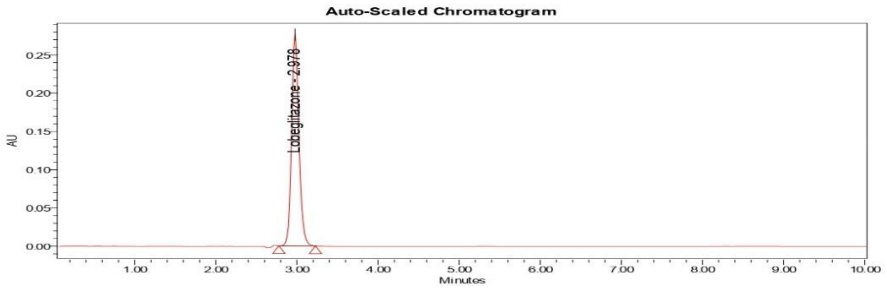


Figure no: - 7 Linearity graphs

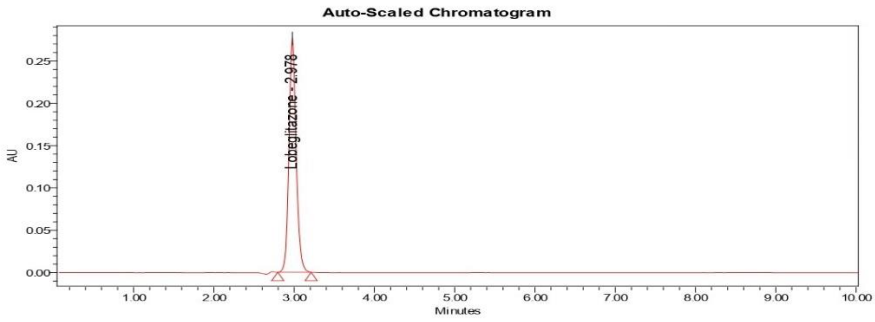
2. precision



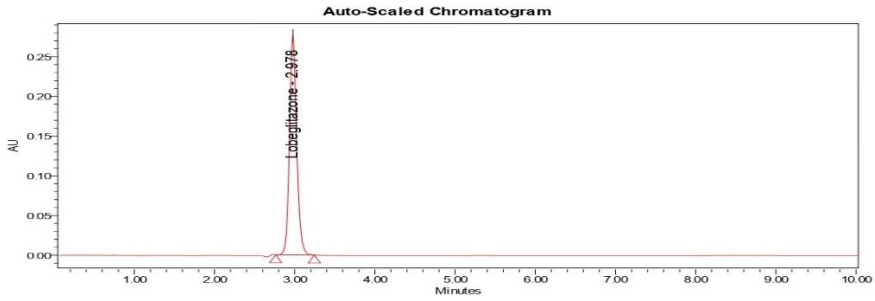
First injection of precision



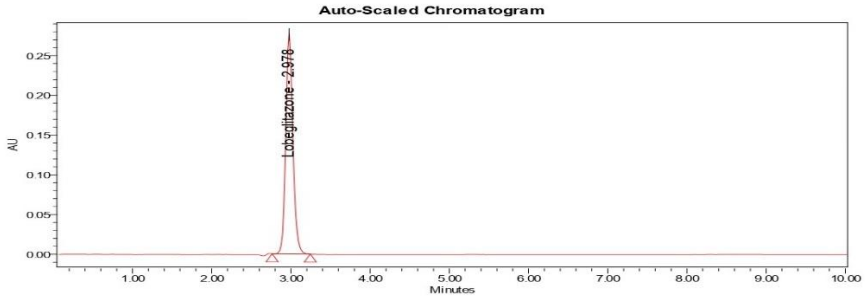
Second injection of precision



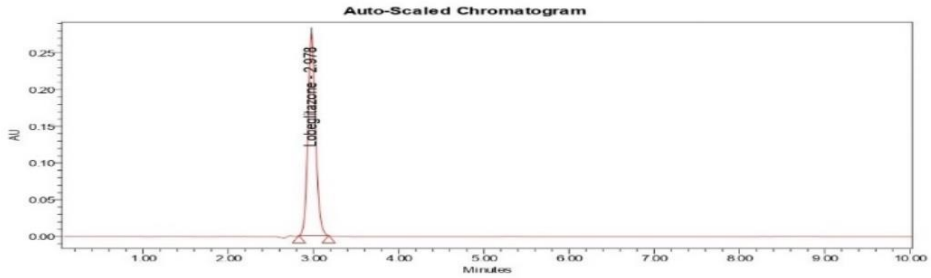
Thirdinjectionof precision



Fourth injection precision



Fifthinjectionofprecision



Sixth injection of precision

Table 3: Precision (Repeatability) results of lobeglitazone

	Name	RT	Area	Height	USPPlateCount	USPTailing
1	Lobeglitazone	2.978	1714738	274871	5413.47	1.14
2	Lobeglitazone	2.978	1716576	274929	5411.33	1.14
3	Lobeglitazone	2.978	1710915	274725	5418.63	1.13
4	Lobeglitazone	2.978	1715938	274861	5413.22	1.14
5	Lobeglitazone	2.978	1696553	274053	5440.47	1.14
6	Lobeglitazone	2.978	1714738	274871	5413.47	1.14
Mean		2.30.0	171576.3			
Std.Dev.		1.0	7617.7			
%RSD			0.4			

Table No: 4 Intermediate Precision results of Lobeglitazone

Injection	Day1, Analyst 1	Day2, Analyst 2
Injection-1	1714738	1714628
Injection-2	1716576	1715466
Injection-3	1710915	1710824
Injection-4	1715398	1714287
Injection-5	1696553	1685443
Injection-6	1714738	1713628
Average	**1711576.3**	**1709046.2**
Standard Deviation	**7617.7**	**7213.6**
%RSD	**0.4**	**0.4**

3. Accuracy

i) Accuracy -50%

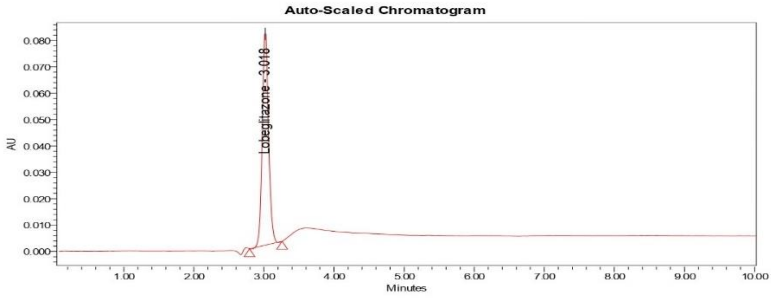


Figure no:9 Accuracy data 50 %

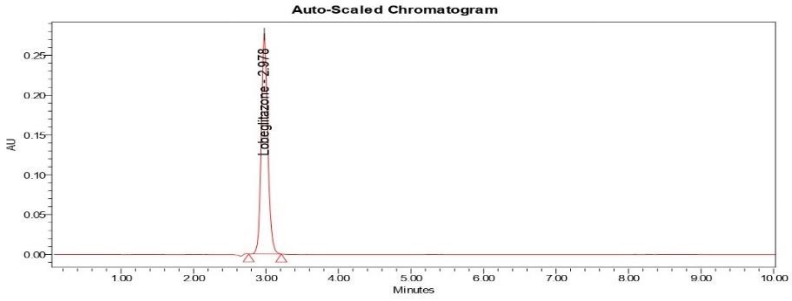


Figure no: 10 Accuracy 100% data

Accuracy - 150%

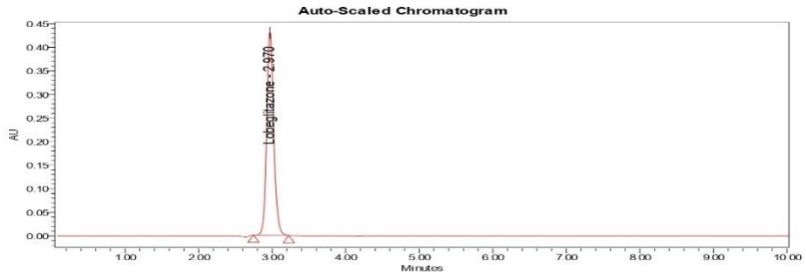


Table No: 5 Accuracy results of Lobeglitazone

Concentration (At specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean %Recovery
50%	499371.6	5	4.99	99.90	99.80
100%	1712552.4	10	9.88	98.96	
150%	2837496.2	15	15.08	100.56	

LIMIT OF DETECTION (LOD):

Formula: $\mathbf{LOD = 3.3X\frac{\sigma}{S}}$

Where

σ - Standard deviation (SD)

S - Slope

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 65 µV

Signal Obtained from LOD solution : 195 µV

S/N = 195/65 = 3.01

LIMIT OF QUANTIFICATION (LOQ):

Formula: $\mathbf{LOQ = 10X\frac{\sigma}{S}}$

Where

σ - Standard deviation

S - Slope

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 65 µV

Signal Obtained from LOQ solution : 650µV

S/N = 650/65 = 10.00

Table No: 6 Summary data of validation parameters

S.NO	VALIDATION PARAMETERS	ACCEPTANCE CRITERIA	RESULTS
1.	Linearity	The correlation coefficient should be NLT 0.999	R^2=0.999
2.	Accuracy	The %Recovery at each level should be NLT 80.0% and NMT 120% of the amount added	%Recovery – 99.80
3.	Precision	The %RSD of peaks obtained from the 6 replicate injections should be NMT 2.0%	%RSD- 0.4
4.	LOD	-	0.523
5.	LOQ	-	1.58

Conclusion:

A Novel, fast, RP-HPLC strategy was created and approved for the assessment of Lobeglitazone in unadulterated and Drug dose structure by utilizing Potassium dihydrogen phosphate support and acetonitrile in the proportion 0f 70:30. The proposed strategy was approved according to ICH rules and the technique was viewed as basic, direct, exact, exact, strong and reproducible. Therefore, it can be concluded that the newly developed RP-HPLC method was statistically evaluated and can be used for routine analysis of lobeglitazone in its pharmaceutical dosage form and pure form. The DOE approach is a flexible method for speeding up the development of a method by reducing the number of trial and error runs required. The method that was suggested was found to be quick, accurate, precise, specific, durable, and cost-effective.

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