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QBD-ASSISTED METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF LOBEGLITAZONE IN BULK AND ITS FORMULATION BY UV- SPECTROSCOPY

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ABSTRACT

This study presents the development and validation of a UV spectrophotometric method for the estimation of lobeglitazone in bulk and pharmaceutical formulations by QbD (central composite design). Lobeglitazone, a potent anti-diabetic agent, was analyzed using UV spectroscopy due to its simplicity, cost-effectiveness, and widespread availability. Till date, there is no UV-visible spectrophotometric method reported using the AQbD approach. The method was optimized by selecting methanol as solvent, wavelength 240nm and 1.134 of maximum absorption. Validation parameters such as linearity, precision, accuracy, ruggedness, and robustness were evaluated according to ICH guidelines. The method exhibited good precision over the concentration range, with correlation coefficient values are less than 2%. The LOQ and LOD of the established method were found to be 0.83µg/ml, 0.70µg/ml and 2.54µg/ml,2.14 µg/ml. The measured linearity of the calibration curve ranges from 2 µg/ml to 14 µg/ml, with a R^2 of 0.997. %RSD of system suitability, inter-day and intra-day precision value of 0.11&0.14% respectively. Accuracy, with Mean recovery was found to be 100,99.6,99.2 respectively for 50,100 & 150% with their %RSD values being 1.04-0.39. The robustness was evaluated for change in wavelength and results were found to be 0.17&0.23 respectively. The %RSD for ruggedness were found to be 0.26&0.18. Overall, the development of UV

spectrophotometric method proved to be simple, accurate, precise, and robust for the estimation of lobeglitazone in bulk and its formulation with application of Quality by Design.

Keywords: Lobeglitazone, UV spectroscopy, QbD, Method Validation, Design Expert 12.0.

INTRODUCTION:

Lobeglitazone is an anti-diabetic drug from the thiazolidinedione class, primarily functioning as an insulin sensitizer by binding to and activating Peroxisome Proliferator-Activated Receptors (PPAR) gamma in fat cells **[1]**. This activation enhances insulin binding at these cells, resulting in reduced blood sugar levels, lowered hemoglobin A1C (HbA1C) levels, and improved lipid and liver profiles. Unlike [DB01132], a dual PPAR agonist affecting both PPAR-alpha and PPAR-gamma, lobeglitazone is a selective PPAR-gamma agonist. It was approved by the Ministry of Food and Drug Safety in South Korea in 2013 and underwent postmarketing surveillance until 2019 **[2]**. However, it is not approved for diabetes management by the FDA in the USA, Health Canada, or the European Medicines Agency. Additionally, lobeglitazone, part of the glitazone class of antihyperglycemic agents, exhibits potent agonist activity for PPAR-alpha alongside its effects on PPAR-gamma **[2]**.

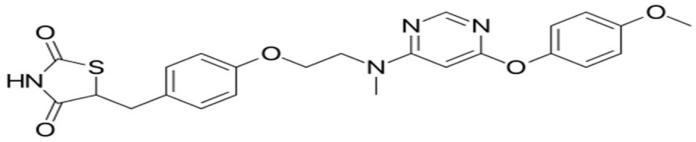

Figure 1: Structure of lobeglitazone

The concept of Quality by Design (QbD) is used in pharmaceutical process development to ensure predefined product quality **[5]**. According to ICH Q2(R1), QbD is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management".

Applying QbD principles to analytical methods offers similar benefits to those seen in manufacturing processes and product development **[4]**. Numerous researchers have incorporated QbD principles into the analytical method development process. The AQbD approach enables the development of robust and cost-effective analytical methods applicable at any stage of the product lifecycle **[5]**. Regulatory authorities now allow changes to analytical methods without revalidation if the AQbD approach was used during method development.

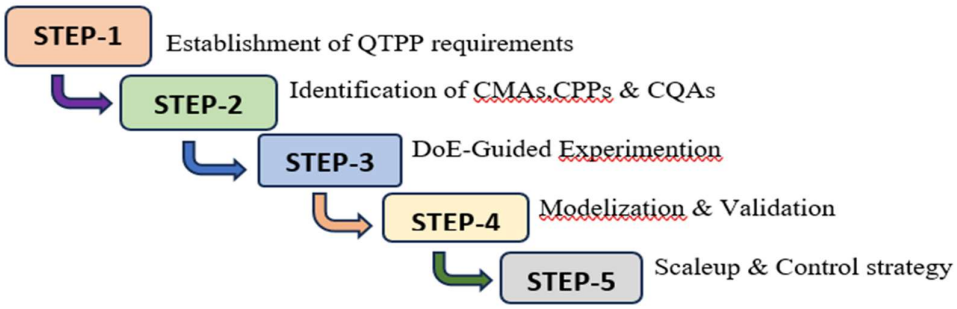

Figure 2: Steps in Analytical QbD

An analytical target profile (ATP) for the procedure is set as the first step in the AQbD approach. ATP is a set of metrics that represent the performance of the analytical approach and establishes its objective **[6]**. Analytical methods can have different performance characteristics according to the ICH Q2(R1) standards on validation of analytical processes **[7]**. In light of this, an ICH guideline Q2 (R1)-based UV spectrometric approach can be created using QbD. The current investigation's objective was to establish an analytical quality by design (AQbD) approach-based, straightforward, fast, reliable, flexible, and affordable UV spectrometric method for the estimation of lobeglitazone **[8]**. Implementing the QbD approach to the UV spectrophotometric analytical method will require an understanding of how method input factors affect the spectral shape, absorbance intensity, and absorbance maxima (max), which were examined, crucial parameters for the suggested approach were chosen. The suggested approach was then verified in accordance with ICH principles. ICH Q2(R1) **[9-10]**.

EXPERIMENTAL WORK

MATERIALS AND METHODS

Lobeglitazone Sulfate was obtained as a gift sample from Glenmark pharmaceuticals Ltd. All the reagents and solvents used in the study were of high quality.

INSTRUMENTS

UV Spectrophotometer (Make:Shimadzu, Model No.UV-1800 double beam spectro-photomete, Make: LABINDIA, Model No.UV 3200 UV/VIS Spectrophotometer), Sonicator (Make:Bio-Tecnics India, Model No.2.25L70H , QbD(Design Expert 12.0). Chemicals used were methanol,0.1N NaOH,1N HCl. 17 runs were obtained for three factors (Solvent 1 as NaOH, Sonication time, Solvent 2 as Methanol). Where the responses were observed for Absorbance & Lambda max.

SELECTION OF SOLVENT WITH QbD SOFTWARE

The QbD software was utilized to determine which solvent would be best for dissolving Lobeglitazone Sulfate. With the use of this

software, many experiments were carried out to determine the maximum absorbance of Lobeglitazone Sulfate using various solvents, including 0.1 N HCl, 0.1 N NaOH, acetonitrile, and methanol. In methanol, the maximum absorbance (λ max) was discovered to be at 240 nm. As a result, methanol was chosen as the validation solvent for lobeglitazone sulfate.

END RESULTS OF INDEPENDENT VARIABLES ON λmax

Design Expert 12, a QbD program, was used to examine the impact of independent variables on dependent variables. The duration of sonication and the solvent ratio were chosen as independent variables. It was determined how these factors affected the dependent variables, absorbance and lambda max.

Table 1

	Factor 1	Factor 2
Run	A:Methanol	B:Sonication time
	%	Min
1	100	20
2	95	15
3	90	10
4	90	15
5	95	20
6	100	10
7	95	15
8	95	10
9	95	15
10	90	20
11	100	15

PREPARATION OF SOLUTION

The solubility of lobeglitazone was determined in various solvents, including methanol, 0.01N hydrochloric acid, 0.01N sodium hydroxide, and distilled water. Lobeglitazone was discovered to be most soluble in methanol. For this reason, typical stock and working solutions are made with methanol. To create the stock solution, 10 mg of lobeglitazone were dissolved in 10 µg/ml of methanol to produce a concentration of 1000µg/ml. The stock solution was diluted to provide a standard working solution of 10µg/ml, which was utilized for the UV spectrum's.

DETERMINATION OF ABSORBANCE MAXIMA(λmax)

A 10 µg/ml concentration was prepared by pipetting 1ml of the working standard solution above into a 10 µg/ml volumetric flask and adding methonal to bring the volume up to the required level. After that, the was scanned in the 200-400 nm range using methanol as a blank in a UV spectrophotometer. It was found that 240 nm was the wavelength corresponding to maximal absorbance.

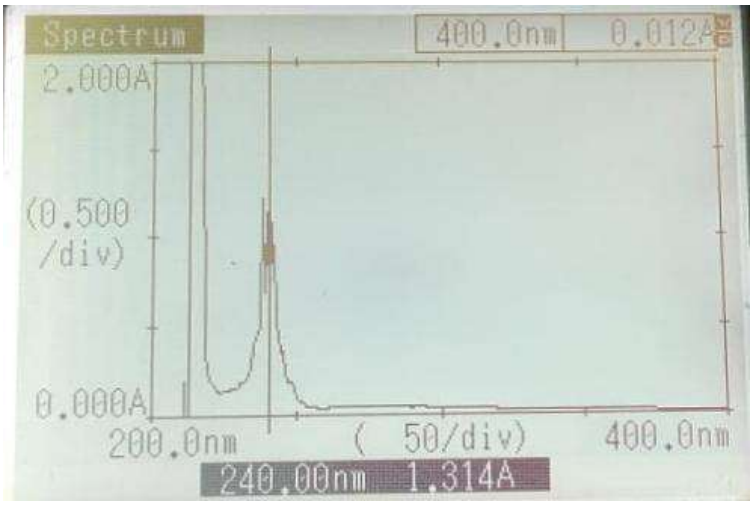

Figure 3: Spectra of Lobeglitazone

METHOD VALIDATION

The selected critical parameters should comply with the method performance characteristics of an analytical method to achieve the analytical target profile. The ICH guidelines Q2(R1) outline various performance characteristics for an analytical method. Therefore, it is appropriate to validate a spectrophotometric analytical method according to these ICH guidelines using the selected critical parameters to implement an AQbD approach. Consequently, the developed method is further validated as per ICH guidelines Q2(R1). The characteristics studied include system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), and limit of quantification (LOQ).

LINEARITY

According to ICH guidelines, the linearity of an analytical procedure confirms that test results are directly proportional to the concentration (amount) of the analyte in the sample. For the linearity study, seven solutions of varying concentrations (2,4,6,8,10,12 and 14µg/ml) were prepared in methanol from a standard stock solution of methanol. The absorbance of each solution was measured at 240 nm in triplicate. A calibration curve was then created by plotting absorbance against concentration. The percentage relative standard deviation (% RSD) and the correlation coefficient were calculated through regression analysis.

PRECISION

According to ICH guidelines, the precision of an analytical procedure assesses the closeness of results obtained from multiple measurements of the same homogeneous sample. Both repeatability (intra-day precision) and intermediate precision (inter-day precision) were evaluated to demonstrate the method's precision. For repeatability, six replicates of a 14 µg/ml concentration were analyzed on the same

day, and the % RSD of the assay results was calculated. For intermediate precision, six replicates of the 14 µg/ml concentration were analyzed over the consecutive day, and the % RSD was calculated.

ACCURACY

The accuracy of an analytical procedure indicates the closeness of results to the true value. Accuracy for was lobeglitazone determined through a recovery study. Known amounts of Drug product solution (4 µg/ml) were spiked with a standard stock solution at different levels (50%, 100%, and 150%). These solutions were reanalyzed for drug content, and triplicate sets of each level were prepared. The recovery of the sample and % RSD were calculated.

ROBUTNESS

This technique was established by analyzing data at two distinct wavelengths (240+/-1) and noting the absorbance, If there are no changes in absorbance. It indicates that the method is robust.

RUGGEDNESS

It was determined by analysing six samples of same batch of lobeglitazone separately in two sets. Days of experiment, the individuals performing them and the equipment used should be different from each other. The overall standard deviation and %RSD should be less than 2%.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The LOD is the smallest amount of analyte detectable but not necessarily quantifiable, while the LOQ is the smallest concentration that can be determined with accuracy and precision. LOD and LOQ for lobeglitazone were determined using the residual standard deviation of the response and slope method as per ICH guidelines. The calibration curve from the linearity study was used, with LOD calculated using the equation (3.3 x σ)/S and LOQ using (10 x σ)/S, where σ is the standard deviation of the response and S is the slope of the calibration curve.

RESULTS AND DISCUSSIONS

UV SPECTROSCOPIC METHOD VALIDATION

LINEARITY

In order to generate the calibration curve, we first plotted **(Figure 3)** the peak area versus the concentration of the analyte. **Table 2** presents the linearity test findings that were obtained.

Table 2: linearity study for lobeglitazone

S. No.	Concentration	Absorbance
1	2	0.123
2	4	0.254
3	6	0.432
4	8	0.606
5	10	0.798
6	12	0.975
7	14	1.185

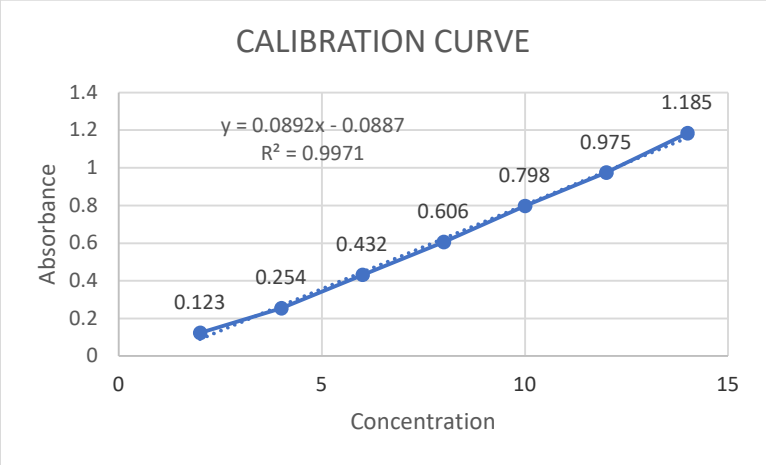

Figure 4: Linearity curve of Lobeglitazone

PRECISION

The approach was evaluated with a stock solution of tablets for this parameter. Six assay runs were performed on the same day for intraday precision investigations, and on different days for interday precision studies. These are the analysis's findings.

Table 3: precision study for lobeglitazone

INTRA DAY		INTER DAY	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
14ppm	**1.125**	**14ppm**	**1.124**
14ppm	**1.126**	**14ppm**	**1.125**
14ppm	**1.125**	**14ppm**	**1.128**
14ppm	**1.124**	**14ppm**	**1.126**
14ppm	**1.127**	**14ppm**	**1.128**
14ppm	**1.128**	**14ppm**	**1.127**
Mean	**1.125833**	**Mean**	**2.252667**
SD	**0.001344**	**SD**	**0.003266**
RSD	**0.119352**	**RSD**	**0.144983**

ACCURACY

The accuracy of an analytical procedure reveals how closely the recorded value matches the reference value. Another calibrated approach was used to carry out the recovery trials. The recorded findings were deemed to be adequately good, as follows:

Table 4: Accuracy study for lobeglitazone

Concentration	Drug sample	Drug product	%Recovery	Mean	SD	RSD
50%	**0.122**	**0.255**	**99.22**	**100.00**	**1.04**	**1.04**
	0.121	**0.256**	**101.17**			
	0.121	**0.253**	**99.60**			
100%	**0.255**	**0.256**	**100.39**	**99.60**	**1.37**	**1.37**
	0.255	**0.253**	**98.02**			
	0.256	**0.254**	**100.39**			
150%	**0.43**	**0.254**	**98.82**	**99.21**	**0.392164665**	**0.395293732**
	0.431	**0.251**	**99.20**			
	0.432	**0.252**	**99.60**			

ROBUSTNESS

The method's robustness was assessed by altering the wavelength by ±1 nm from 239nm to 241nm, and the results were satisfactory.

Table 5: Robustness study for Lobeglitazone

Concentration	Absorbance at 239	Absorbance at 241
10ppm	0.798	0.797
10ppm	0.796	0.795
10ppm	0.797	0.796
10ppm	0.795	0.794
10ppm	0.796	0.794
10ppm	0.794	0.796
Mean	0.796	0.795333
SD	0.001414	0.001211
RSD	0.177638	0.234902

RUGGEDNESS

Ruggedness was achieved by testing the Lobeglitazone sulfate sample using various tools and analysts. The findings were calculated in terms of %RSD.

Table 6: Ruggedness study for Lobeglitazone

Instrument name – Shimadzu		Instrument name- Lab India	
Day-1		Day-2	
Concentration	Absorbance	Concentration	Absorbance
10ppm	0.792	10 ppm	0.796
10 ppm	0.794	10 ppm	0.797
10 ppm	0.796	10 ppm	0.798
10 ppm	0.797	10 ppm	0.795
10 ppm	0.792	10 ppm	0.796
10 ppm	0.793	10 ppm	0.799
Average	0.794	Average	0.796833
SD	0.002098	SD	0.001472
RSD	0.264231	RSD	0.184731

LIMIT OF DETECTION & LIMIT OF QUANTIFICATION

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations LOD = 3.3 × σ/s and LOQ = 10 × σ/s, respectively. σ is the standard deviation of the drug response regions, regarded as a measure of noise, and S is the slope of the calibration plot. The LOD and LOQ were recorded and confirmed to be within the limitations.

Table 7: LOD & LOQ study for Lobeglitazone

Based on residual SD of Regression line		Based on y-intercept	
LOD	LOQ	LOD	LOQ
0.838598065	2.541206259	0.708745	2.147711

OPTIMIZATION FORMULATION BY THE CENTRAL COMPOSITE DESIGN METHOD BY USING DESIGN EXPERT SOFTWARE 12

Table 8: Effect of independent variables on absorbance

	Factor 1	Factor 2	Response 1	Response 2
Run	A: Methanol	B: Sonication time	Absorbance	Lambda Max
	%	Min		
1	100	20	1.2	238
2	95	15	0.103	239
3	90	10	0.08	241
4	90	15	0.091	240
5	95	20	1.04	238
6	100	10	0.167	240
7	95	15	0.103	239
8	95	10	0.09	240
9	95	15	0.103	239
10	90	20	0.94	235
11	100	15	0.18	239

ANOVA for Reduced Quadratic model

Table 9: Response 1: Absorbance

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.99	4	0.4973	597.25	< 0.0001	significant
A-Methanol	0.0317	1	0.0317	38.05	0.0008	
B-Sonication time	1.35	1	1.35	1617.91	< 0.0001	
AB	0.0075	1	0.0075	8.99	0.0241	
B^2	0.6029	1	0.6029	724.07	< 0.0001	
Residual	0.0050	6	0.0008			
Lack of Fit	0.0050	4	0.0012			
Pure Error	0.0000	2	0.0000			
Cor Total	1.99	10				

Fit Statistics

Std. Dev.	0.0289		R^2	0.9975
Mean	0.3725		Adjusted R^2	0.9958
C.V. %	7.75		Predicted R^2	0.9857
			Adeq Precision	58.2179

The Predicted R^2 of 0.9857 is in reasonable agreement with the Adjusted R^2 of 0.9958, with a difference of less than 0.2.

Adequate Precision evaluates the signal-to-noise ratio. A ratio greater than four is preferred. Your ratio of 58.218 suggests a good signal. This model can help you explore the design space.

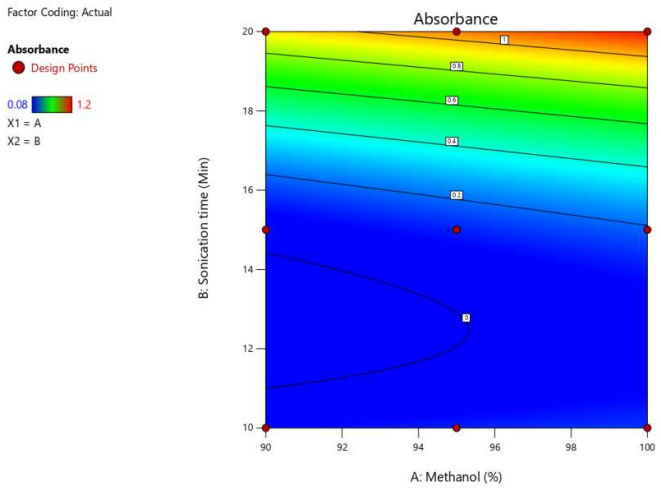

Figure 4: Effect independent variables on dependent variables

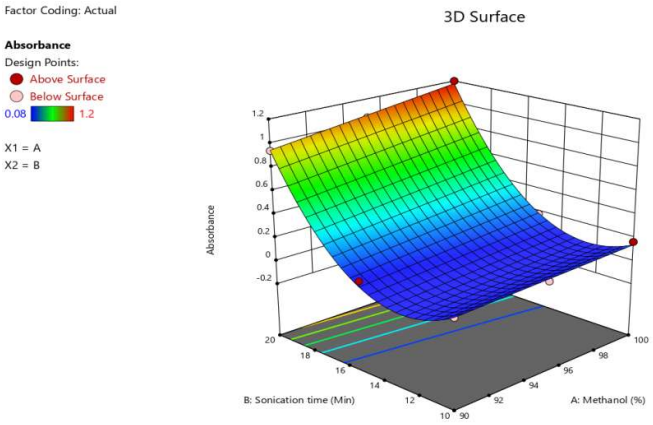

Figure 5: 3D surface plot for ANOVA results

ANOVA for 2FI model

Table 10: Response 2: Lambda Max

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	20.83	3	6.94	11.93	0.0039	significant
A-Methanol	0.1667	1	0.1667	0.2862	0.6092	
B-Sonication time	16.67	1	16.67	28.62	0.0011	
AB	4.00	1	4.00	6.87	0.0344	
Residual	4.08	7	0.5823			
Lack of Fit	4.08	5	0.8152			
Pure Error	0.0000	2	0.0000			
Cor Total	24.91	10				

Fit Statistics

Std. Dev.	0.7631		R^2	0.8364
Mean	238.91		Adjusted R^2	0.7662
C.V. %	0.3194		Predicted R^2	0.2277
			Adeq Precision	11.5907

The Predicted R^2 of 0.2277 differs from the Adjusted R^2 of 0.7662 by more than 0.2. This could imply a huge block effect or an issue with your model and/or data. Model reduction, response transformation, outliers, and so on are all important considerations.

All empirical models should be tested through confirmation runs.

Adeq Precision evaluates the signal-to-noise ratio. A ratio greater than four is preferred. Your ratio of 11.591 shows a sufficient signal. This model can help you explore the design space.

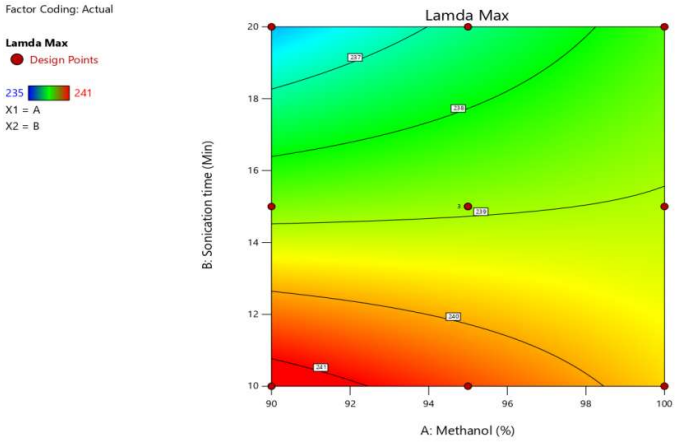

Figure 6: Effect of independent variables on dependent variables

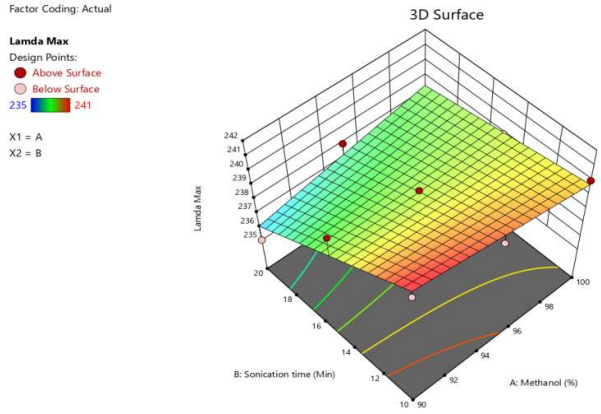

Figure 7: 3D surface plot for ANOVA result

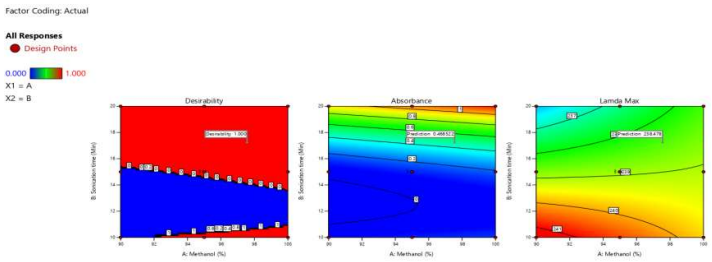

Figure 8: Plot to determine the desirability of the method

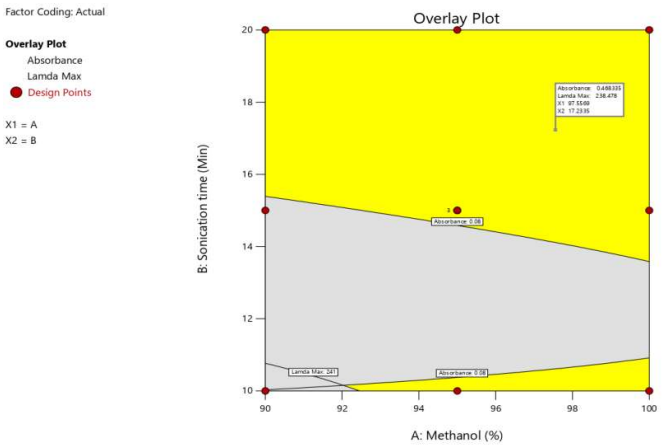

Figure 9: Overlay plot to determine sonication time and ratio of solvents

DISCUSSION

Many UV-spectrophotometric methods have been developed for various drugs for their estimation in bulk and pharmaceutical formulations. In recent years, researchers have also developed UV-spectrophotometric methods

for estimation of drugs in nano formulations, and demonstrated QbD approach during their developmental process. This work has been designed based on the past developed methods for estimation of QBD assisted method development validation for estimation of lobeglitazone in bulk and its formulation by UV spectroscopy nano formulations. All the results of validation parameters lie within the acceptable limits. The method's specificity and selectivity were accomplished due to the absence of interference from widely used excipients.

Obtained LOD and LOQ values of 0.83µg/m**l**, 0.70µg/ml shows the minimum concentration which can be detected by the method. Results of precision studies were found to be %RSD <2, which is an acceptable value. Ruggedness values were 0.264 and 0.184 of day 1, day 2.

CONCLUSION

Current method utilizing various Analytical target parameters of solvent and sonication time, the responses of absorbance and lambda maximum were found to be optimum at 240nm.Validation parameters performed according to ICH Q2(R1), ICH Q13 & Q14 guidelines are meeting the criteria. Observed range (2 µg/ml-14 µg/ml) was well within the limit of regression analysis(r 2=0.997).Current method with the application of AQbD parameters was successful in accomplishing the solvent and time invested in method development for the same. Hence the method can be utilized for regular quality control analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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