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Liquid Chromatographic – Estimation Of Lobeglitazone Sulphate In Bulk And Marketed Formulation

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ABSTRACT
A new thiazolidinedione (TZD) based peroxisome proliferator-activated receptor (PPAR) agonist, lobeglitazone sulphate has the potential to be used therapeutically to treat type 2 diabetes mellitus. Linearity, accuracy, and precision criteria were employed to design and verify the RP-HPLC technique. to create an RP-HPLC technique that is straightforward, precise, accurate, and specific for estimating lobeglitazone sulphate. Linearity, accuracy, and precision factors were employed in the development and validation of HPLC chromatographic techniques. The acetonitrile-methanol (75:25, v/v) mobile phase was used to create the HPLC technique at a wavelength of 248 nm. 5 to 25 ug/ml was the linearity range that was observed, and the correlation value was 0.9997.
The findings were statistically analysed under the ICH Q2 (R1) requirements, and the created approach was precise with a %RSD value not higher than 2 for both intra-day (1.54%) and inter-day (1.07%) precision. The findings in this paper demonstrated the invention and validation of a straightforward, quick, and economical RP-HPLC technique for lobeglitazone sulphate quantification. The developed method can be applied alone or in conjunction with the specified drugs for routine analysis and quality control.
Keywords: *Lobeglitazone sulphate, RP-HPLC, ICH Q2 (R1) guidelines, Precision, %RSD.*

INTRODUCTION

The metabolic disease known as type 2 diabetes mellitus (T2DM) is progressive and persistent. Its long-term impacts on the body include β-cell malfunction and insulin resistance[1].

The condition known as type 2 diabetes is intricate and multifaceted, with several underlying processes. Consequently, there are several methods for managing the illness[2]. Oral anti-diabetic drugs (OADs) come in several types, but the Thiazolidinedione (TZD) family is notable since it focuses mainly on insulin resistance[2]. By stimulating the proliferator-activated receptor γ (PPARγ), TZDs enhance insulin sensitivity as well as lipid and glucose metabolism[3]. The two drugs that are most frequently prescribed to treat type 2 diabetes and enhance glycemic control are rosiglitazone and pioglitazone, which both raise insulin sensitivity. Due to the risks connected with cardiovascular disease and bladder cancer, their use has lately declined. The newly developed thiazolidinedione "lobeglitazone" (Chong Kun Dang Pharmaceutical Corporation, Seoul, Korea)[5] is a better and safer alternative to the present TZDs. It works similarly to pioglitazone in terms of glycemic control, although at lower doses. Furthermore, encouraging safety results from clinical studies have addressed some of the problems associated with earlier TZDs. Since July, it has been used as a Type 2 Diabetes treatment in Korea due to its pleiotropic effects in pre-clinical and clinical testing[6,7]. Lobeglitazone is a pharmacophore made composed of an ethoxy-benzyl N-methylamino group coupled to a 2,4-thiazolidinedione group. Its chemical name is 5-[4-(2-{[6-(4-Methoxy}phenoxy)-pyrimidin-4-yl] methyl-amino}-ethoxy)-benzyl]. Hydrosulphuric acid-thiazolidine-2,4-dione[8]. Under the brand name Lobg (Glenmark Pharmaceuticals Ltd.), lobeglitazone pills are sold on the market. To study the pharmacokinetics of Lobeglitazone in rats, Jong-Hwa Lee and colleagues created a validated technique utilising tandem mass spectrometry and liquid chromatography[9]. A bioanalytical approach was created by Gulhane et al. to estimate the amount of Lobeglitazone in human plasma[10]. According to the review of the literature, no technique for determining Lobeglitazone in pharmaceutical dosage forms has been published yet, except for a few bioanalytical approaches. Analytical techniques are always changing to remain straightforward, dependable, affordable, repeatable, and most importantly, accurate and precise in response to changing requirements. The objective of our research was to create an isocratic RP-HPLC technique that is fast, reliable, selective, sensitive, and accurate for measuring Lobeglitazone (LOBG 0.5 mg) in tablet dosage forms. The test technique was verified by the use of ICH recommendations[10]. The drug content of the LOBGL in pharmaceutical goods was ascertained using the following metrics: linearity,

accuracy, precision, specificity, Limit of Detection (LOD), and Limit of Quantification (LOQ).

MATERIALS AND METHODS

The following chemicals and instruments were used for the analytical method development and validation.

Active pharmaceutical ingredients

Lobeglitazone sulphate and LOBG 0.5 mg tablet

Table I: Chemical solvent and materials used for the analytical method development and validation

Sr No.	Solvent	Grade	Manufacturer
1.	Acetonitrile	HPLC	Merk Life Science Pvt Ltd, chemical
2.	Methanol	HPLC	Merk Life Science Pvt Ltd, chemical

Apparatus and Instruments

Table II: Instrument and equipment used for the analytical method development and validation studies

Sr No.	Name of instruments	Make and Model
1.	HPLC Software	HPLC Jasco- Extrema ChromNav
2.	UV-Vis Spectrophotometer Software	Shimadzu UV-1800 UV probe
3.	Ultra sonicator	Life Care Equipment
4.	Electronic Weighing Balance	Shimadzu (sensitivity 0.001 gm)

Analytical method development for Lobeglitazone sulphate by HPLC Method

Determination of solubility for HPLC analysis

A quantity of standard drug was dissolved in different solvents like water, methanol and acetonitrile. UV method was employed for quantitative estimation of solubility. Selection of suitable solvent

Acetonitrile: Methanol was employed as the solvent in a 75:25 v/v ratio. The solvent was selected based on solubility experiments performed on several solvents and a research of solubility choice solvents.

Preparation of standard stock solution

A 100 mL volumetric flask containing 100 mg of Lobeglitazone sulphate was precisely measured before being diluted with acetonitrile and sonicated to the appropriate strength, yielding a concentration of 1000 µg/mL, to create the standard stock solution. Using acetonitrile, an aliquot of 10 mL of the above standard stock solution was diluted up to the required concentration with acetonitrile in a volumetric flask of 100 mL, giving a concentration of 100 µg/mL (sub-stock solution).

preparation of sample stock solution

Twenty-five tablets were precisely weighed and crushed into a fine powder for the sample stock solution. A volume equivalent to 10 mg was calculated, transferred, diluted with acetonitrile, and sonicated for 15 minutes in a volumetric flask with a total volume of 100 mL. To prepare the solution with a 100 µg/mL concentration, 1 mL of the sample stock solution mentioned above was transferred into a 10 mL volumetric flask and diluted with acetonitrile.

Selection of Detection Wavelength

The standard solution of 10 µg/mL of Lobeglitazone sulphate was prepared and scanned over the 400-200nm range. After the scan was completed, it showed maximum absorbance at 248nm, so the detection wavelength was selected as 248nm.

Chromatographic Conditions

Table III: Chromatographic Conditions

Sr no.	Specification	Description
1	Equipment	JASCO Extrema lC system-4000
2	Software	CHROMNAV
3	Column	Inertsil C_{18} (250 x 4.6 mm, ID 5µm)
4	Wavelength	248 nm
5	Column temperature	25°C
6	Flowrate	1.0 mL/min
7	Injection volume	10 µL
8	Run time	10 min

9	Mobile phase	Acetonitrile: methanol (75:25)
10	Diluent	Acetonitrile
11	Elusion mode	Isocratic

Selection of Mobile Phase

A range of solvents was screened to get the drug's sharp, well-resolved, and symmetrical peak. Preparations of standard solution and chromatographic conditions for the analysis were the same as discussed above in 4.5.2 and 4.5.3 respectively with respective changes in mobile phase composition.

Various trials were conducted to select a suitable mobile phase for HPLC method development.

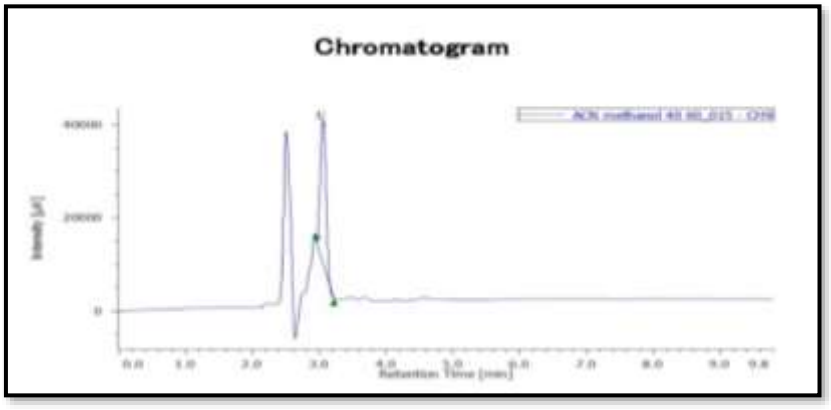

Figure 1:Trial 1- Methanol: ACN in the ratio 40:60v/v

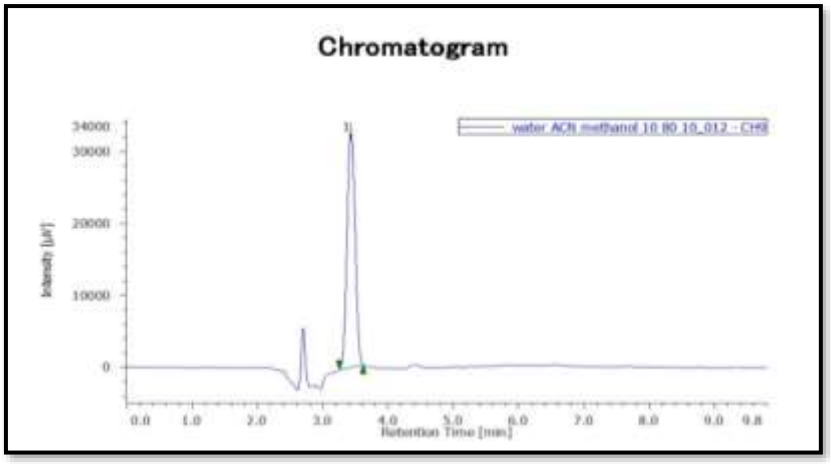

Figure 2:Trial 2- Water: ACN: Methanol in the ratio 10:80:10v/v

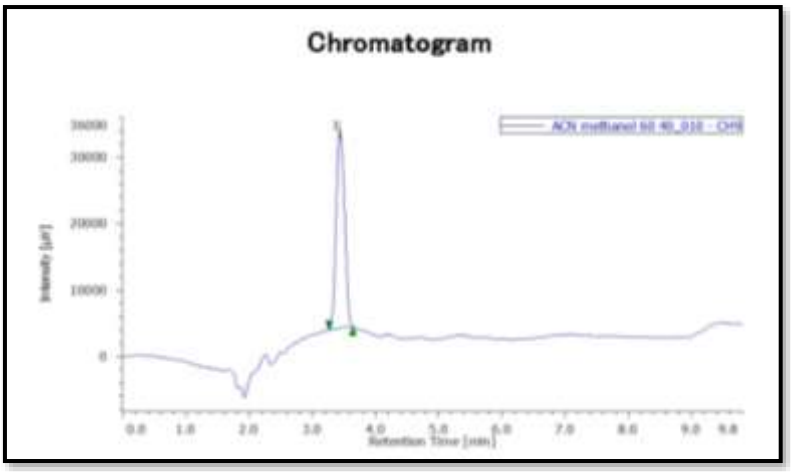

Figure 3:Trial 3- ACN: Methanol in the ratio 60:40v/v

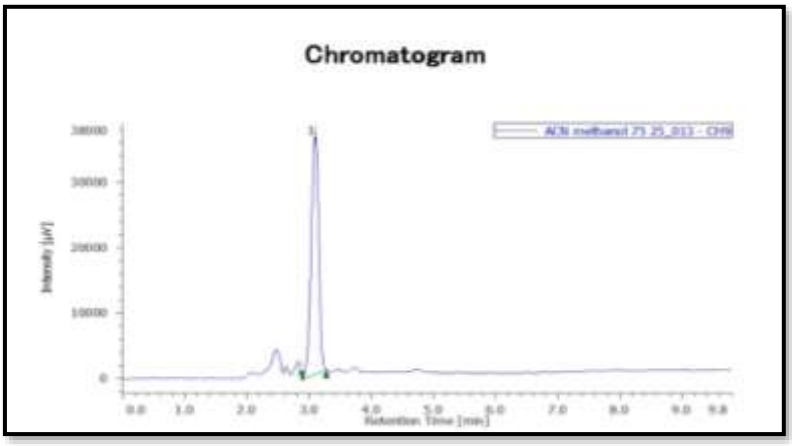

Figure 4:Trial 4- ACN: Methanol in the ratio 75:25v/v

For mobile phase selection, peak symmetry, tailing factor, peak height, No. of theoretical plates etc. were considered. So number of trials were performed for the selection of the mobile phase.

Analytical Method Validation for Lobeglitazone sulphate by HPLC Method

Specificity study

The following solutions were prepared and injected to prove that the method developed is specific.

Blank solution- Diluent used as a blank

Standard solution- Preparations of standard solution for the analysis were the same as directed in the method of analysis procedure

Sample solution- Preparations of standard solution for the analysis were the same as directed in the method of analysis procedure

Check the identification of drug peak and interference study.

Linearity

Accurately weighed 10mg of the drug, transferred in a 10 ml volumetric flask and diluted it with the solvent up to the mark to get the concentration of 1000 µg/mL. from the above solution, the linear response of the drug was determined over the range of 5-25 µg/mL.

The calibration curve was plotted of peak area vs. Concentration. The correlation coefficient and Y-intercept of the linearity curve were calculated.

Accuracy

The accuracy of the method was determined by spiking with a known amount of drug X to result in sample solutions with the following concentrations of drug 80%, 100% and 120% relative to the working concentration in triplicate according to the method of analysis and analyzed as per the method. The % recovery was calculated.

Precision

Repeatability

A standard solution of 25 µg/ml was prepared and injected 6 times into the system. A chromatogram was recorded and the %RSD of the Peak Area was calculated.

Intermediate Precision

The intermediate precision was calculated by measuring the responses of a standard peak on the same day and another day of the same solution concentration.

LOD & LOQ

Six sets of linearity concentrations were analysed and LOD & LOQ were calculated using the following equations as per ICH guidelines, based on the response and slope of a regression equation.

Robustness

The following parameters have been changed one by one and their effect on system suitability test.

- Change in flowrate ±0.2 ml/min
- Change in temperature ±5°C
- Change in wavelength ±2 nm

RESULTS AND DISCUSSION

System suitability parameters

System suitability tests were carried out as part of the validation procedure to make sure the HPLC system was operating dependably and consistently. Six duplicate injections of the

standard solution at a concentration of 10 µg/mL were produced for each validation parameter.

Table IV: Results of System Suitability

SR NO.	PARAMETER	RESULTS
1.	Retention time	2.9
2.	Theoretical plates	3492
3.	Linearity range (µg/mL)	5-25
4.	Detection limit(µg/mL)	1.0438
5.	Quantification limit(µg/mL)	3.1630
6.	Regression data: Slope	24275
7.	Regression data: Intercept	3134.69
8.	Regression data: Correlation coefficient	0.9997

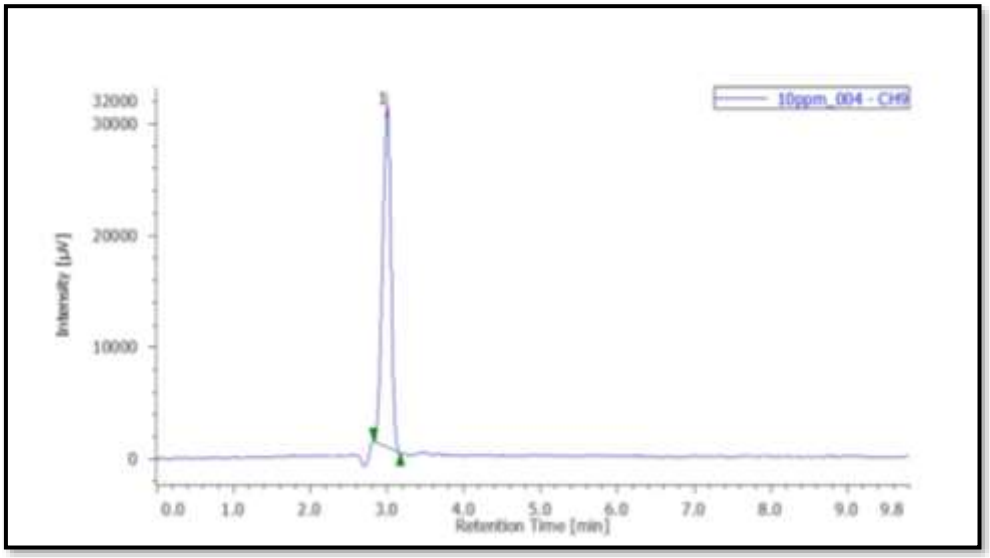

Figure 5: Standard chromatogram data of Lobeglitazone sulphate

Linearity

A series of dilutions were made using the standard stock solution to evaluate the linearity of the medication Lobeglitazone sulphate. The dilutions covered a concentration range of 5–25 µg/mL.

Table V: Linearity data for Lobeglitazone sulphate

Concentration	Area
0	0
5	116558
10	232981
15	355771
20	482515
25	605504

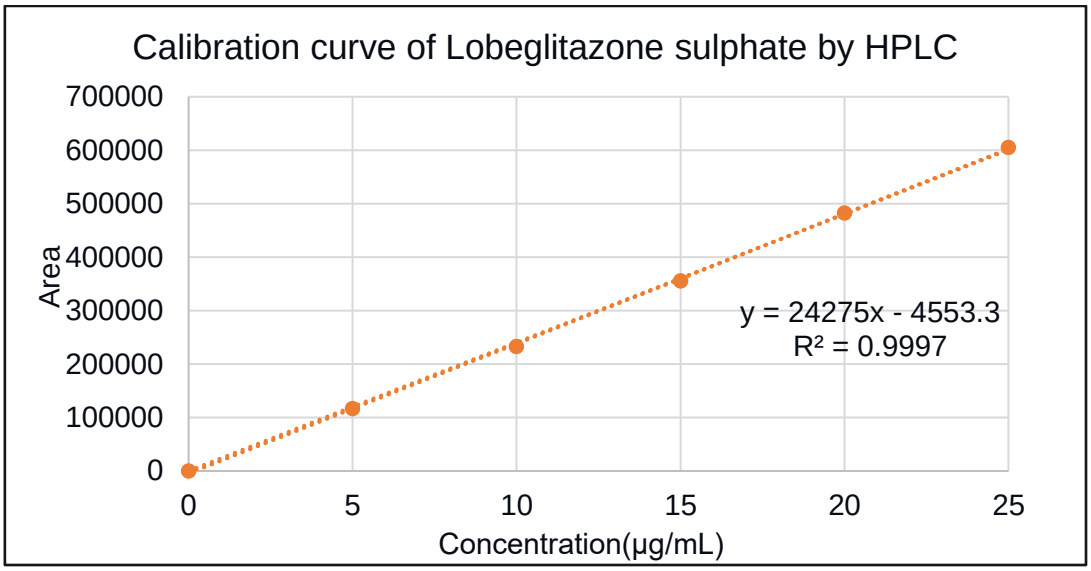

Figure 6: calibration curve of lobeglitazone sulphate by HPLC

Accuracy

Percentage drug accuracy of three different concentrations 80%, 100%, and 120%.The mean recovery percentage was then calculated based on the results obtained from the recovery studies.

Table VI: Results of accuracy by HPLC method

Level	Peak area	Calculated conc	% recovery	Mean conc	Standard deviation	%RSD
0.8	392711	15.99	100.70%	16.0567	0.09006	0.56092
	396816	16.1591				
	393459	16.0208				
1	483515	19.7306	98.91%	19.8912	0.13926	0.70008

	489193	19.9646				
	489532	19.9785				
1.2	589412	24.093	101.83%	24.2198	0.20721	0.85554
	589760	24.1074				
	598293	24.4589				

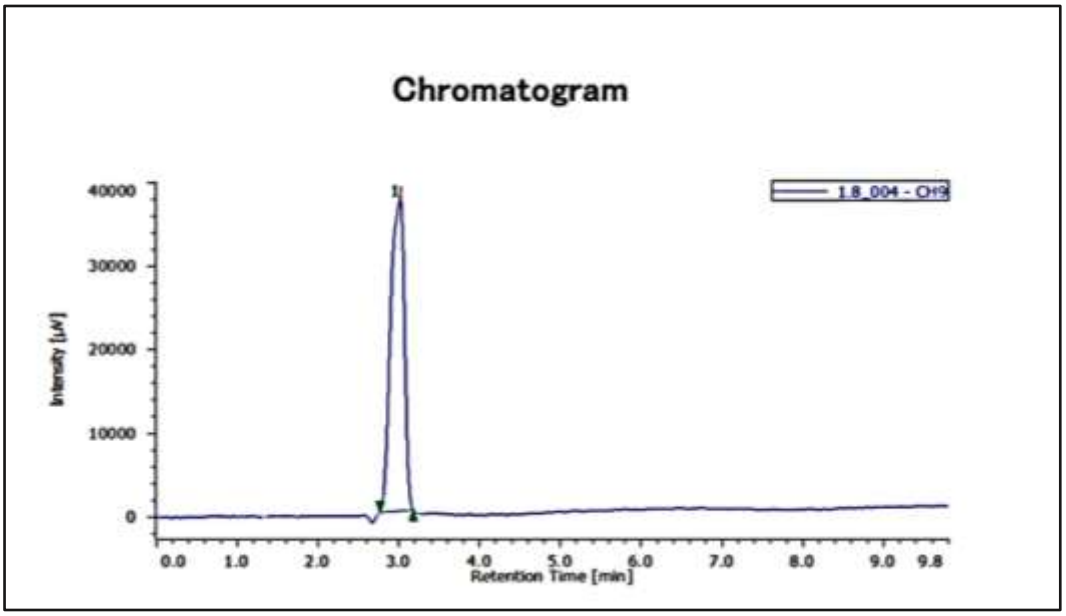

Figure 7: chromatogram of lobeglitazone sulphate for accuracy [80%]

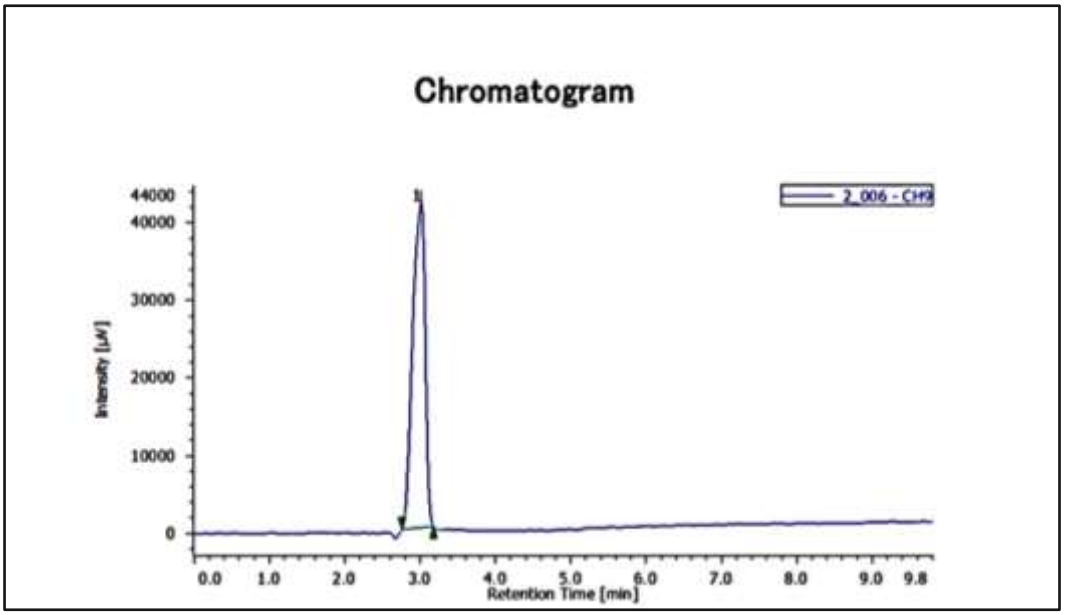

Figure 8: chromatogram of lobeglitazone sulphate for accuracy [100%]

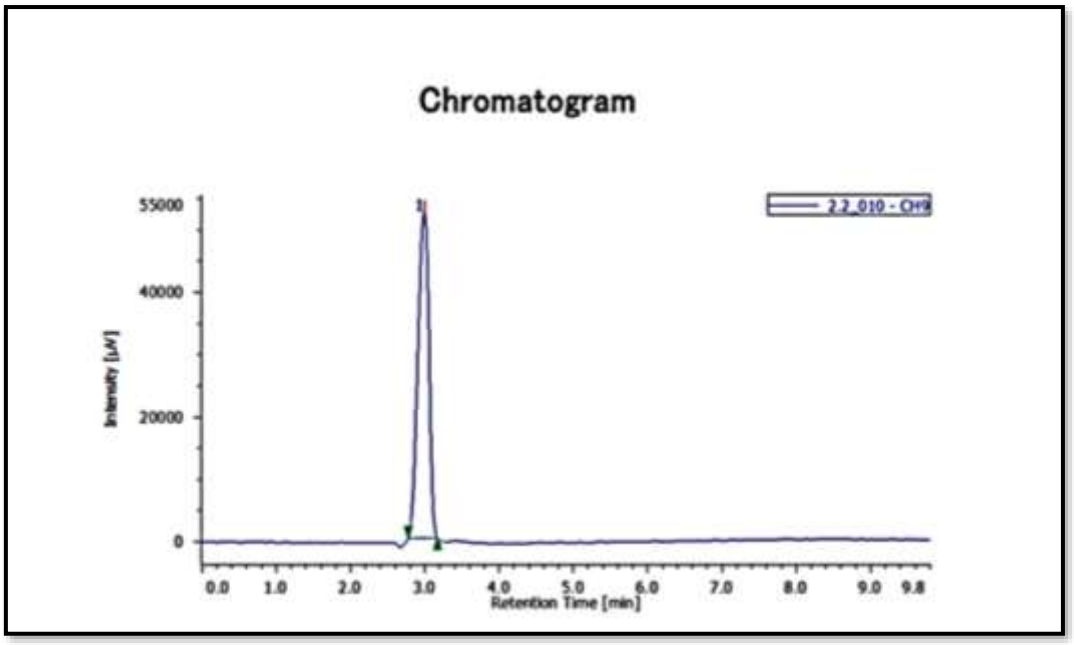

Figure 9: chromatogram of lobeglitazone sulphate for accuracy [120%]

PRECISION

Precision was analyzed by the method intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three days). A precision study was performed by injecting six times of standard solution. Results are expressed as %RSD.

Table VII: Results of intraday precision by HPLC method

Sr no.	Sample name	Area	Theoretical plate	Tailing factor	Retention time
1	Test_1	612347	3158	1.28	2.997
2	Test_2	607466	3144	1.28	3
3	Test_3	608704	3119	1.29	3.003
4	Test_4	621269	3167	1.29	3.003
5	Test_5	595887	3195	1.28	3
6	Test_6	598405	3122	1.29	3

MEAN	607346	3150.83	1.29	3.00
SD	9294.82	28.83	0.01	0.00
%RSD	1.53	0.91	0.43	0.08

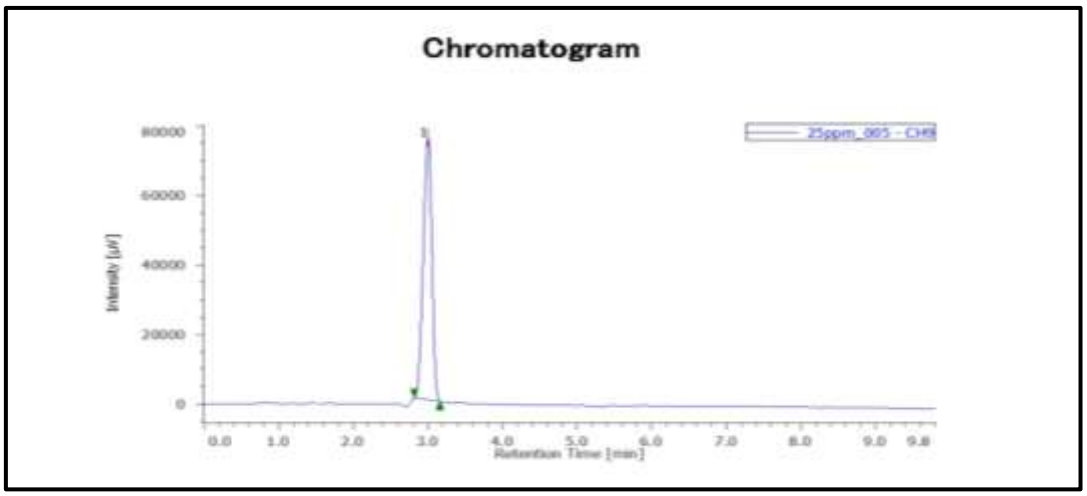

Figure 10 : chromatogram of Lobeglitazone sulphate for intraday precision

Table VIII: Results of interday precision by HPLC method

Sr no.	**Sample name**	**Area**	**Theoretical plate**	**Tailing factor**	**Retention time**
1	Test_1	610431	2983	1.29	3
2	Test_2	616939	2939	1.29	3
3	Test_3	623576	2933	1.3	3.003
4	Test_4	628400	2941	1.3	3.007
5	Test_5	613736	2947	1.3	3.003
6	Test_6	620424	2926	1.3	3.003
MEAN		618918	2944	1.3	3.0026666
SD		6582.89	20.02	0.01	0.00
%RSD		1.06	0.68	0.40	0.09

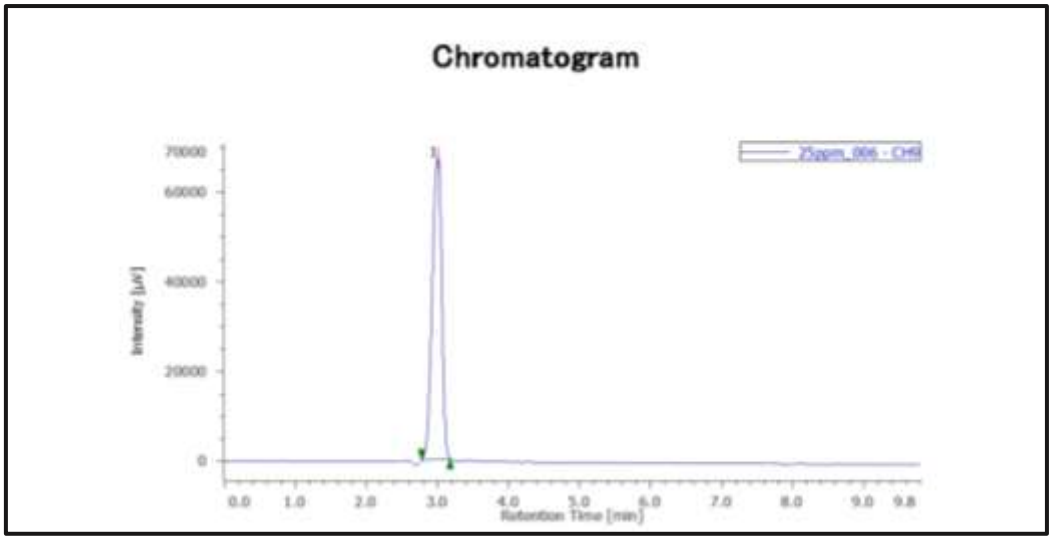

Figure 11: chromatogram of Lobeglitazone sulphate for intraday precision

Robustness

The following parameters have been changed one by one and their effect on system suitability test.

- Change in flowrate ±0.2 ml/min
- Change in temperature ±5°C
- Change in wavelength ±2 nm

Table IX: Robustness data of Lobeglitazone sulphate by HPLC

SR NO.			1	2	3	MEAN	SD	%RSD
FLOW RATE	1.2ml/min	AREA	264038	262483	262330	262950	945.05	0.36
		RT	2.483	2.5	2.497	2	0.01	0.36
		NTP	2650	2612	2680	2647	34.08	1.29
	0.8ml/min	AREA	400065	397126	394085	397092	2990.1	0.75
		RT	3.71	3.68	3.69	4	0.02	0.41
		NTP	2331	2347	2354	2344	11.79	0.50
TEMP	20°C	AREA	320433	321808	319058	320433	1375.00	0.43
		RT	2.963	3.003	3	3	0.02	0.75
		NTP	2696	2640	2657	2664	28.71	1.08
	30°C	AREA	317713	318487	316418	317539	1045.38	0.33

		RT	2.987	2.913	2.9	3	0.05	1.60
		NTP	2923	2922	2948	2931	14.73	0.50
WAVELENGTH	246nm	AREA	315849	319085	314687	316540	2279.05	0.72
		RT	2.903	2.903	2.903	3	0.00	0.00
		NTP	3206	3280	3254	3247	37.54	1.16
	250nm	AREA	310266	306416	305333	307338	2592.61	0.84
		RT	2.897	2.897	2.9	3	0.00	0.06
		NTP	3387	3470	3484	3447	52.43	1.52

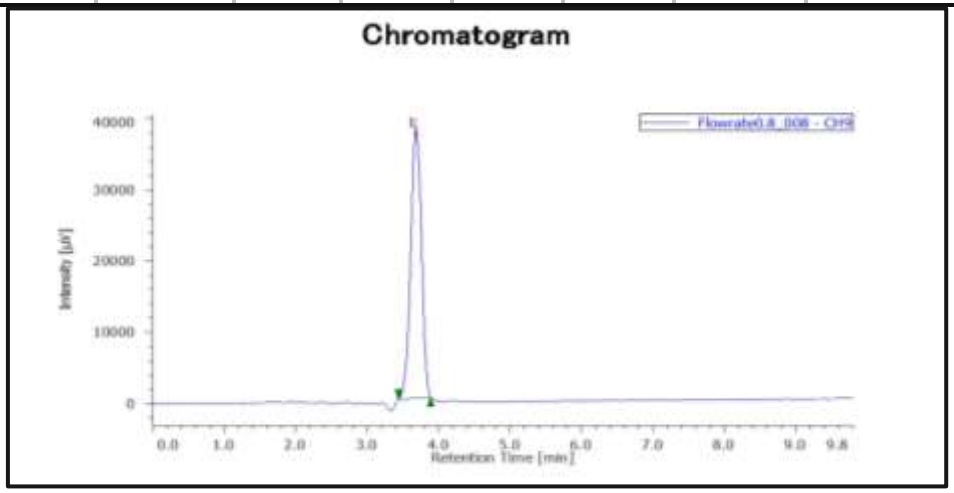

Figure 12: Chromatogram of Lobeglitazone sulphate for robustness (0.8 ml/min)

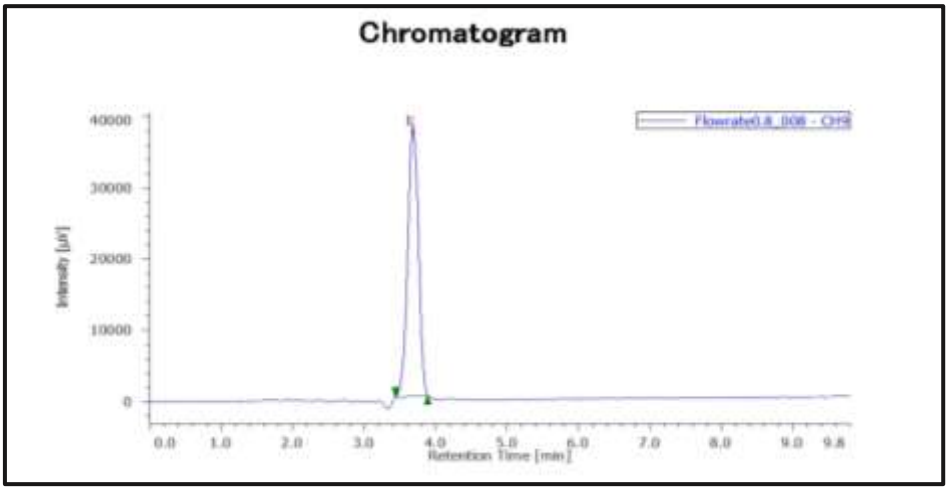

Figure 13: Chromatogram of Lobeglitazone sulphate for robustness (1.2 ml/min)

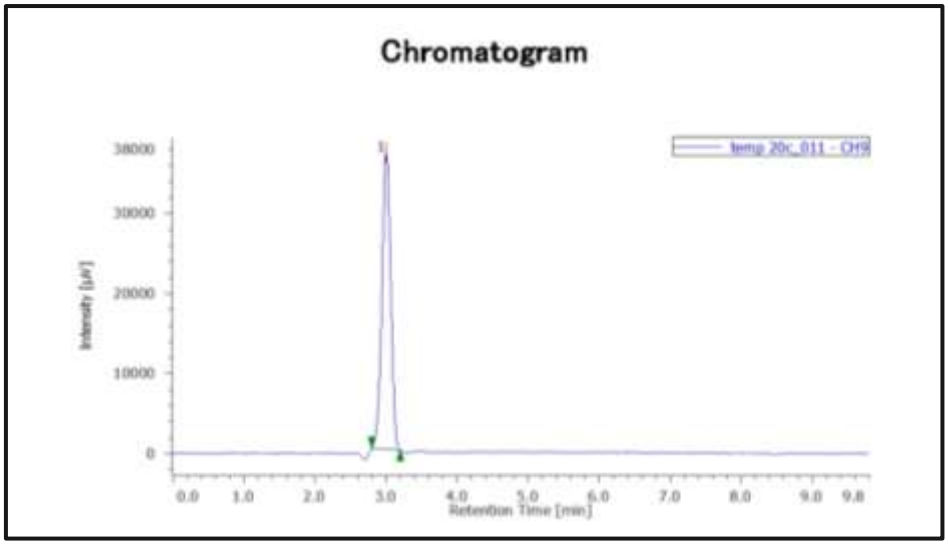

Figure 14: Chromatogram of Lobeglitazone sulphate for robustness (20°C)

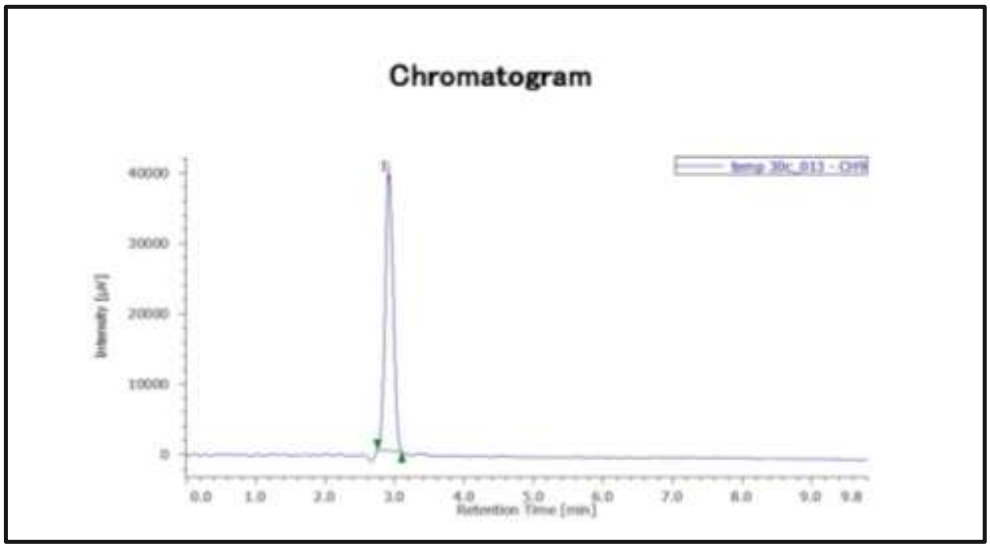

Figure 15: Chromatogram of Lobeglitazone sulphate for robustness (30°C)

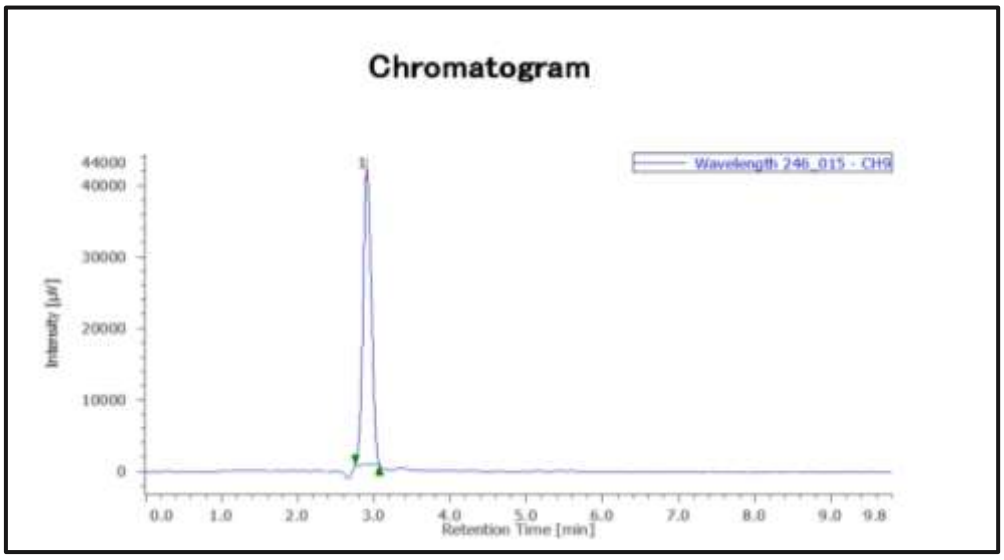

Figure 16: Chromatogram of Lobeglitazone sulphate for robustness (246nm)

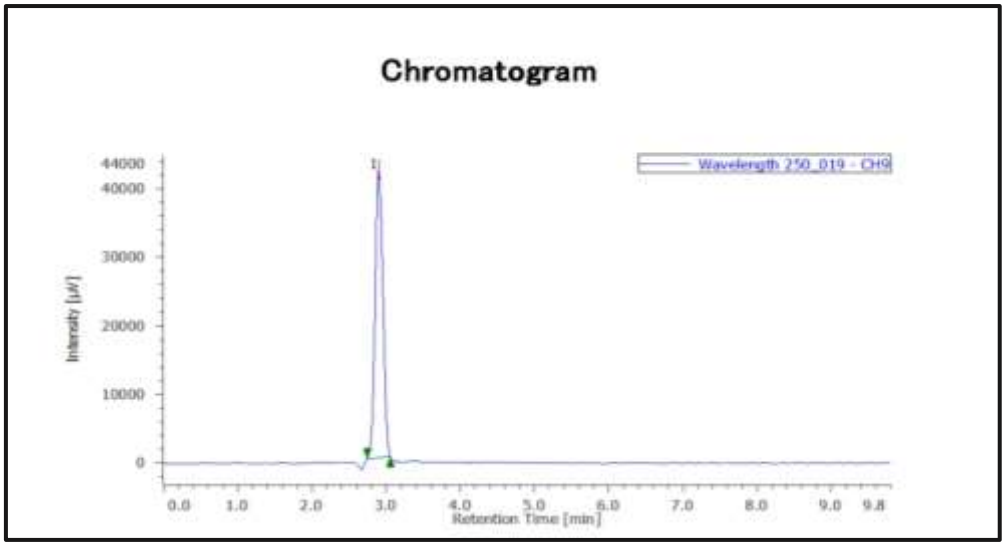

Figure 17: Chromatogram of Lobeglitazone sulphate for robustness (250nm)

CONCLUSIONS

The effective development and validation of the HPLC technique for the quantification of Lobeglitazone sulphate was carried out using a JASCO Extrema lC system-4000 HPLC model. Using an Inertsil C_{18} (250 x 4.6 mm, ID 5µm) column and a single wavelength, the devised approach is innovative for drug determination. The injection volume is 10 µL. Lobeglitazone sulphate's method which complies with ICH guidelines was found to be straightforward, accurate, sensitive, quick, reliable, and affordable. In a short amount of time, the analytical conditions were created with acceptable resolution. Less than 2% was discovered to be the upper limit of the percentage RSD for all metrics. This shows that the technique created is appropriate for measuring Lobeglitazone sulphate in labs and for quality assurance needs.

Conflicts of interest

The authors declare that they do not have any conflicts of interest. The authors are keenly responsible for the content and preparation of this article.

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