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RP-HPLC method for the estimation of Lobeglitazone sulphate in pharmaceutical dosage form

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Abstract

A simple, rapid, accurate and precise RP-HPLC method was developed and validated for the determination of Lobeglitazone sulfate in table dosage form. Chromatographic analysis of the drug was achieved on Shimadzu HPLC comprising of LC- 20 AD binary gradient pump, a variable wavelength programmable SPD-20A detector and SCL system controller. Kromasil column (250 mm x 4.6 mm, 5 µ) as stationary phase with mobile phase consisting of 1% glacial acetic acid and acetonitrile in the ratio of 30 : 70 v/v. The method showed a good linear response in the concentration range of 10-60 µg/ml with correlation coefficient of 0.9990. The flow rate was maintained at 1.0 ml/min and detection was carried out at 254 nm. The retention time was 4.211 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity and sensitivity. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the determination of lobeglitazone sulfate in tablet formulation.

Keywords: Lobeglitazone sulfate; RP-HPLC; Tablet dosage form; Validation; ICH

1. Introduction

Currently a global epidemic, type 2 diabetes (T2D) is a metabolic illness primarily marked by decreased insulin production and action [1,2]. The thiazolidinedione class includes lobeglitazone as one of its orally delivered anti-diabetic medications [3], treats type 2 diabetic mellitus (T2DM) [4-6]. The heterocyclic nucleus thiazolidine-2,4-dione has been intensively studied for use in the development of innovative medications for a number of pathophysiological illnesses, including melanoma, diabetes complications, cancer, arthritis, and inflammatory diseases [7]. When compared to people without type 2 diabetes, patients with it have an almost double the death risk [8,9]. At the transcriptional level, lobeglitazone sulphate predominantly affects PPAR-gamma (peroxisome proliferator-activated receptor-gamma) receptors and drives some important metabolic pathways related to glucose and lipid metabolism [10]. Other thiazolidinedione class drugs like troglitazone, which was discontinued in 2000 due to liver toxicity, rosiglitazone, and pioglitazone, which showed cardiovascular risks and bladder cancer [11,12], led Chong Kun Dang Pharma in South Korea to introduce lobeglitazone sulfate, as compared to the therapeutically utilised TZD-PPAR gamma activators pioglitazone and rosiglitazone, the substance is said to have at least a 1.11- and 16.6-fold greater affinity respectively [13,14] which was approved by the South Korean Ministry of Food and Drug Safety in 2013 [15]. When broken down chemically, lobeglitazone sulfate is composed of 5 -(4-(2-(((6-(4-Methoxyphenoxy) pyrimidine-4-yl) (methyl)amino) ethoxy) benzyl) thiazolidine-2,4-dione; sulfuric acid. Numerous publications on lobeglitazone sulfate clinical trials and bioanalysis have been published utilizing various analytical methods, including Liquid chromatography with tandem mass spectrometry (LCMS/MS)[16-23], high-performance liquid chromatography (HPLC), thin layer chromatography, ion pair HPLC[24-25]. Out of these analytical methods HPLC[26-60] is most widely used technique when compare to UV spectrophotometric methods[61-89] in quantitative analysis of drugs. In spite of various HPLC methods available

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for the estimation of lobeglitazone sulfate , still there is a need of a economical and rapid HPLC method for its estimation in dosage forms. The present study, a new RP-HPLC method was developed for estimation of lobeglitazone sulfate in pharmaceutical formulation, which shown high reproducibility, sensitivity and economical. The developed method was validated as per ICH guidelines[90]

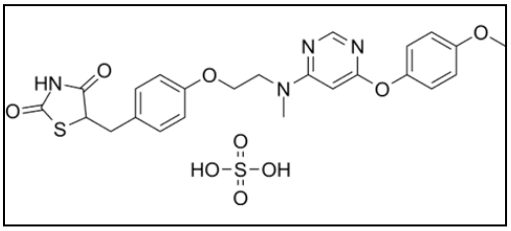

Figure 1 Structure of lobeglitazone sulfate

2. Materials and methods

2.1. Instrumentation

Chromatographic separation was performed on a Shimadzu LC-20AD with binary pump, and a variable wavelength UV detector SPD 20A is used. Rheodyne injector fitted with a 20µL loop was used and data were recorded and analysed using LC solution software. An Kromasil C18 G column (250*4.6 mm i.d,5-µm particle)was used.'LC solution' software was used to collect and process the data. Ultra sonicator (Citizen ultra sonicator) was used for sonicating the drug and sample solution.Digital weighing balance (SHIMADZU AUX 220) used for weighing.

2.2. Chemicals and Reagents

Lobeglitazone sulfate pure drug (purity 99.1%) was obtained as gifted sample from Hetro Drugs Ltd. Marketed formulation Atazor capsules (label claim 300 mg) was procured from local market. HPLC grade water , Acetonitrile was from MERCK India Ltd. HPLC grade methanol was from standard reagent Pvt Ltd Hyderabad. Analytical grade glacial acetic acid was from SD Fine chemicals Mumbai, India. Nylon membrane filters 0.2 µm and 0.45 µm were from PALL life sciences Mumbai, India.

2.3. Chromatographic conditions

The chromatographic system used for method development and validation includes Shimadzu LC-20AD with binary pump, and a variable wavelength UV detector SPD 20A is used. Rheodyne injector fitted with a 20µL loop was used and data were recorded and analysed using LC solution software. An Kromasil C18 G column (250*4.6 mm i.d,5-µm particle)was used.. A mixture of 1% glacial acetic acid and acetonitrile in a 20: 80 v / v ratio was found to be the ideal mobile phase for the ideal chromatographic analysis of Lobeglitazone sulfate. The solvent mixture was filtered through a 0.22 µ membrane filter and sonicated before use. It is pumped through the column at a flow rate of 1.0 mL / min. The injection volume is maintained in the column at 20 µL and room temperature. The column was balanced by pumping the mobile phase through the column for at least 20 min before injecting the drug solution. The detection was monitored at 254 nm. Run time is set to 10 minutes. Optimized chromatographic conditions are shown in Table 1.

Table 1 Optimized chromatographic conditions

Parameters	Conditions
Stationary Phase (Column)	C_{18} (250 × 4.6 mm i.d.,5µ)
Mobile Phase	Acetonitrile: 1 % glacial acetic acid(70:30,v/v)
Flow rate(ml/min)	1.0 mL/min
Run time(min)	10 min
Column temperature (°C)	Ambient
Volume of injection loop(µL)	20
Detection wavelength(nm)	254 nm

Retention time(min)	4.211

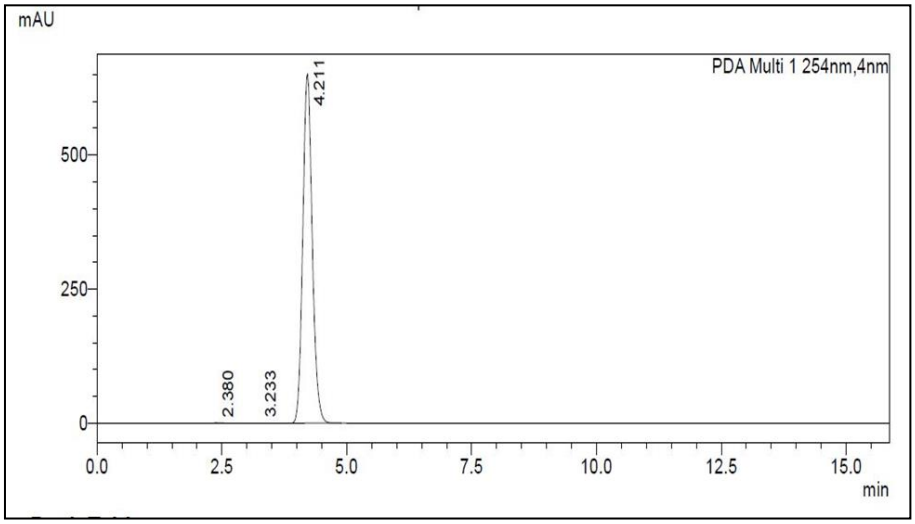

Figure 2 Chromatogram of standard solution of Lobeglitazone sulfate

2.4. Preparation of mobile phase

Mobile phase was prepared by mixing 700 mL of HPLC grade acetonitrile with 300 mL 1% glacial acetic acid (3 ml glacial acetic acid diluted to 300 ml with HPLC grade water). The mobile phase was sonicated for 60 min and filtered through the 0.22 µm membrane filter.

2.5. Preparation of standard stock solutions

The standard stock solutions of 100 µg/mL of the drug were prepared by dissolving 50 mg of pure drug in the mobile phase in a 50 mL volumetric flask and the volume was made up to the mark. Resulting solutions were further diluted with mobile phase to obtain a final concentration of 100 µg/mL and stored under refrigeration. Aliquots of standard stock solutions were put in a 10 mL volumetric flask and diluted up to the mark with mobile phase. In such a way, the final concentrations of the drug were in the range of 10-60 µg/mL

2.6. Preparation of sample solution

Weighed 20 tablet and triturated to fine powder. Dose equivalent to 10 mg of Lobiglitazone Sulfate was transferred to a 100 ml volumetric flask containing mobile phases. This tablet powder was dissolved under sonicated for 20 min to ensure complete solubility. The solution was made up to the mark with methanol and filtered through 0.45µ membrane filter. . Aliquots of this solution within the concentration range were injected into the system and the chromatogram was recorded at 254 nm. The peak area of the drug was calculated and the drug content in the tablet was quantified using the regression equation obtained from the pure sample.

2.7. Method Validation

The developed method was validated as per ICH guidelines by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficients of variation and relative errors of less than 2 % were considered acceptable.

2.7.1. System Suitability Test

Before performing validation experiments, system suitability test (SST) has to be applied to indicate that HPLC system and method are capable of providing data with admissible quality. SST was performed by investigating capacity factor, tailing factor, theoretical plates number, and also relative standard deviation (RSD) of the peak areas.

2.7.2. Stability

Stability was assessed by analyzing QC standard solutions after keeping them at room temperature for 48 hr. Obtained results were investigated as recovery values and compared to the freshly prepared solutions.

2.7.3. Linearity

A stock solution of Lobeglitazone sulfate of 1000 µg/mL was prepared with mobile phase. From it, various working standard solutions were prepared in the range of 10 to 60 µg/ml and injected into HPLC. It was shown that the selected drug had linearity in the range of 10–60 µg/mL. The calibration plot (peak area of Lobeglitazone sulfate versus Lobeglitazone sulfate concentration) was generated by replicate analysis (n=6) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

2.7.4. Accuracy

The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 50%, 100% and 150%, and then comparing the difference between the spiked value (theoretical value) and actual found value.

2.7.5. Precision

The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (30 µg/mL). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra- and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (RSD).

2.7.6. Robustness

Robustness of the proposed method for Lobeglitazone sulfate was carried out by the slight variation in flow rate, analytical wavelength and mobile phase ratio. The percentage recovery and RSD were noted for Lobeglitazone sulfate.

2.7.7. Ruggedness

The test solutions were prepared as per test method and injected under variable conditions. Ruggedness of the method was studied by different analysts.

2.7.8. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formulas:

$$LOD=3.3SD/slope$$

$$LOQ=10SD/slope$$

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y-intercept of regression line, and s is the slope of the calibration curve.

2.7.9. Specificity

The specificity of the proposed method was determined against blank and placebo applications. Here mobile phase was used as blank and excipients like starch, lactose, magnesium stearate were used as placebo.

3. Results and discussion

3.1. Method validation

3.1.1. System Suitability Test

After setting the optimum conditions, system suitability parameters for the developed method were determined and compared with recommended limits. To determine the parameters, the study was performed with standard solution of 20 µg/ml concentration and the results were acquired from six injections. System suitability parameters of the method were demonstrated in Table 2. According to the results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

Table 2 Results of system suitability test (n = 6)

Parameter	Criteria	Result
Capacity factor(k')	$k' > 2$	4.201
Tailing factor (T)	T < 2	1.5
Theoretical plates (N)	N> 2000	4543
% RSD (peak area)	% RSD ≤ 2	0.68

3.1.2. Stability

The sample solution stability was analyzed by injecting the same solution at 0, 12, 24, and 48 h. Identical change was not observed in the developed method. Also, results were found within acceptable limits (% RSD < 2), which are summarized in Table 3.

Table 3 Stability data of Lobeglitazone sulfate (standard solutions)

Time (hr)	Assay(%)	% Difference
Initial	100.08	----
After 12 hr	100.02	0.05
After 24 hr	99.87	0.21
After 36 hr	99.16	0.92
After 48 hr	98.32	1.76

3.1.3. Linearity and sensitivity

Linearity study was performed with calibration standards with 10, 20, 30, 40, 50, and 60 µg/ml concentrations. The standards were injected in triplicate. Calibration curves were obtained by plotting the peak areas against the given concentrations. The calibration curve was evaluated by the determination coefficient. The determination coefficient (R^2) of the calibration curves was 0.999. Therefore, the calibration curve for Lobeglitazone sulfate was found to be linear within the range of 10–60 µg/ml concentrations as shown in Fig.3. The regression equations were calculated from the calibration graphs. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ are given in Table 4.The low values of LOD and LOQ indicates the sensitivity of method.

Table 4 Spectral and statistical data for determination of Istradefyllin by proposed RP-HPLC method

Parameter	Result
Detection wavelength (nm)	254
Linearity range (µg/ml)	10-60
Coefficient of determination (r^2)	0.999
Regression equation (Y[a])	Y= 274186x – 27671
Slope (m)	274186
Intercept (c)	-27671
Limit of detection, LOD (µg/ml)	0.04
Limit of quantitation, LOQ (µg/ml)	0.12

[a]$Y = mx + c$, where x is the concentration (µg/ml).

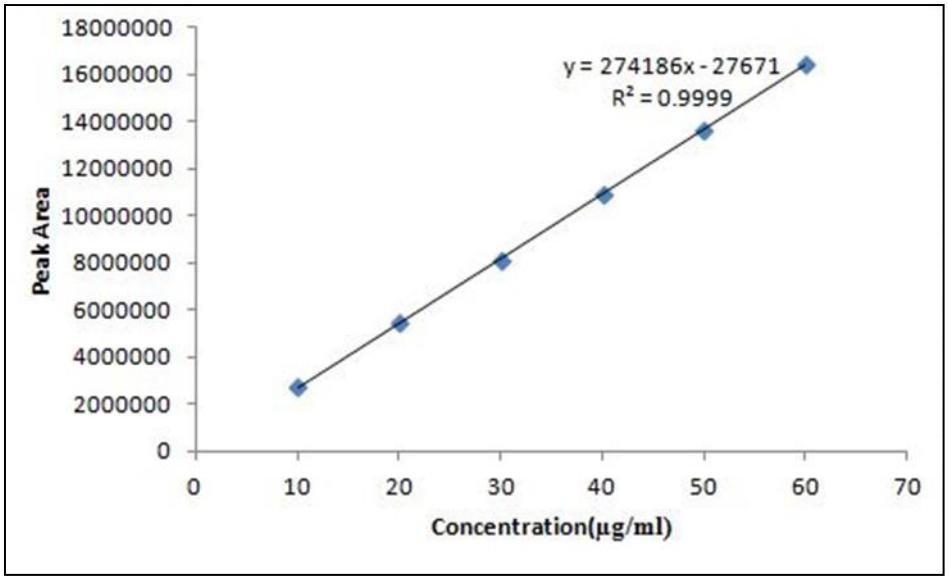

Figure 3 Calibration curve of Lobeglitazone sulfate

3.1.4. Accuracy

To study the reliability, the suitability, and the accuracy of the method, recovery experiments were carried out. Known quantities of the pure drug were added to the preanalyzed sample to make samples at the levels of 50%, 100%, and 150%, and were assayed by the proposed method. Accuracy was calculated as the percentage of recovery. The recovery and relative standard deviation for each of the analytes are given Table 5. From the recovery studies it is evidence that the method is highly accurate and can give excellent results.

Table 5 Accuracy results

% spike Level	Sample	Amount Added (Std)	Amount Found (µg/ml)	% Recovery	Statistical Parameters
50	20	10	9.91	99.1	Mean = 99.1 SD= 0.458 % RSD=0.462
	20	10	9.96	99.6	
	20	10	9.87	98.7	
100	20	20	19.90	99.5	Mean = 98.9 SD= 0.655 % RSD=0.663
	20	20	19.64	98.2	
	20	20	19.80	99.0	
150	20	30	29.73	99.1	Mean = 98.9 SD= 0.529 % RSD=0.535
	20	30	29.49	98.3	
	20	30	29.79	99.3	

3.1.5. Precision

The precision was demonstrated at three levels: repeatability, intermediate precision, and reproducibility (between laboratories' precision). Each level of precision was investigated by 3 sequential replicate of injections of three concentrations of 10, 20 and 30 µg/mL. The precision was expressed as relative standard deviation (RSD) or coefficient of variation (CV). The results of three levels of precision are shown in Table 6. The developed method was found to be precise as the RSD values for repeatability, intermediate precision and reproducibility studies were < 2%, respectively as recommended by ICH guidelines (ICH Q2 (R1), 2005).

Table 6 Precision results

Precision	Results		
	Concentration(µg/mL)	% RSD of Peak area	%RSD of Retention Time
Repeatability	10	0.89	0.02
	20	1.21	0.08
	30	1.11	0.12
Intermediate precision	10	1.42	0.08
	20	0.75	0.06
	30	0.67	0.06
Reproducibility	10	1.64	0.11
	20	0.78	0.17
	30	0.85	0.09

3.1.6. Robustness and ruggedness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1.0±0.1 mL/min), mobile phase composition (± 5 % organic phase) and analytical wavelength (±2 nm). The results are given in Tables 7. The result shown that have the negligible effect on retention time, recoveries and peak area of Lobeglitazone sulfate indicating the developed method is robustness. Ruggedness of the method was carried out by different analysts. The results are displayed in Table 8.There is no variation in peak areas and retention time of Lobeglitazone sulfate from studies carried out by two analysts as indicated by %RSD < 2 gives the method ruggedness.

Table 7 Robustness studies

Parameter	Variation	Observed value			
		% RSD of area	% RSD of R.T	Tailing factor	Theroteical plates(N)
Flow rate	0.9	0.35	0.91	1.5	4541
(m L)	1.1	0.59	0.73	1.5	4534
Mobile Phase	65% methanol	0.42	0.14	1.4	4562
Composition	75 % methanol	0.54	0.13	1.5	4551
Wavelength	252 nm	0.55	0.61	1.5	4545
(nm)	256 nm	0.62	0.74	1.5	4552

Table 8 Ruggedness studies

Analyst	Observed value			
	% RSD of area	% RSD of R.T	Tailing factor(T)	Theroteical plates(N)
Analyst I	0.45	0.63	1.5	4565
Analyst II	0.52	0.72	1.5	4553

3.1.7. Mobile phase stability

The stability of the mobile phase was evaluated, so the mobile phase was stored at 4–8 °C for 1 week. The aged mobile phase was compared using a freshly prepared one. The mobile phase was stable up to 1 week at 4–8 °C.

3.1.8. Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purity of Lobeglitazone sulfate was assessed by comparing the retention times of standard Lobeglitazone sulfate and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected and there were no peaks. There is no interference of blank and placebo on drug peaks hence, the method is specific.

3.1.9. Sample Analysis

The developed and validated method was applied for analysis of capsule formulation contains Lobeglitazone sulfate. The sample was analyzed in triplicate. Analysis results were evaluated using a calibration curve. The amount of Lobeglitazone sulfate in the samples was calculated from calibration curve equation and recovery and RSD values were determined. The results of analysis are given in Table 9. The recoveries were in good agreement with the label claims. The chromatogram obtained was clear as shown in Fig. 4. It was concluded that the method can be applied successfully for the analysis of Lobeglitazone sulfate in tablet dosage form.

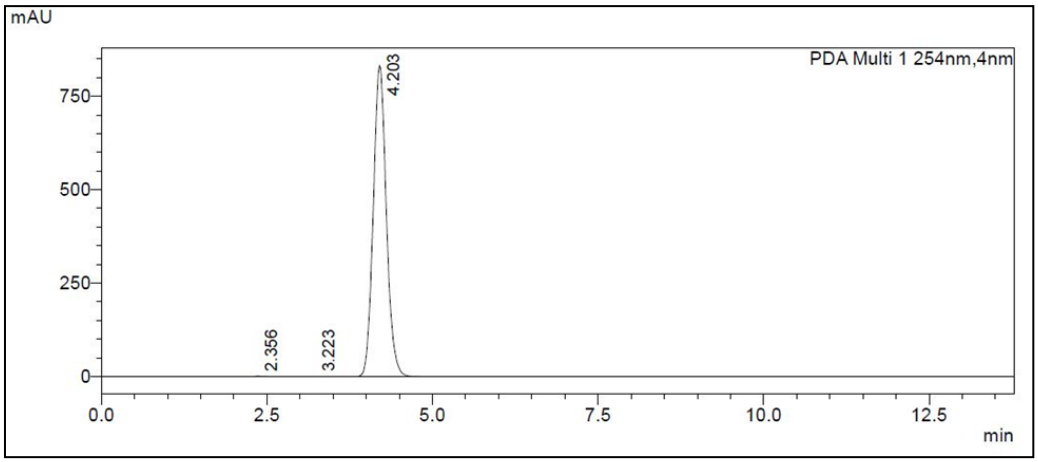

Figure 4 Chromatogram of sample Lobeglitazone sulfate

Table 9 Assay results from commercial formulation

Tablet Formulation	Drug	Labeled Claim (mg)	Amount found* (mg)	% Mean Recovery* ± % RSD
LOBI	Lobeglitazone sulfate	0.5 mg	0.499 mg	99.87 ± 0.94

* Average of five determinations

4. Conclusion

The proposed method for the estimation of Lobeglitazone sulfate was validated as per the ICH guidelines and it is simple, specific and economical. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Lobeglitazone sulfate in pharmaceutical formulations without any interference from the excipient.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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