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Research Article

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Development and Validation of HPLC Method for Accurate Determination of Saroglitazar in Pharmaceutical Preparations

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Abstract An accurate, efficient, and consistent stability indicating RP-HPLC method was created and verified for quantifying Saroglitazar in pharmaceutical formulations. The determination of saroglitazar was conducted using the RP-HPLC method with a C8 chromasil column. The method utilized a mobile phase consisting of a phosphate buffer with a pH of 4.5 and methanol in a ratio of 25:75, v/v. The pH of the mobile phase was changed to 4.5 using ortho-phosphoric acid. Analysis was conducted at a wavelength of 238.2 nm. The linearity was detected within the concentration range of 4-20 µg/mL. The method had a run time of 10 minutes. The mean retention time for saroglitazar was determined to be 7.670 minutes at a flow rate of 1.0 ml/min. The analysis conducted using the proposed method yielded results that were sensitive, simple, reliable, precise, accurate, and resilient. These findings imply a strong agreement with the label claim of the medicine. This approach is suitable for the regular analysis of saroglitazar.

Keywords Saroglitazar, Method Development, RP-HPLC

Introduction

Saroglitazar is a pharmaceutical compound used to treat type 2 diabetes mellitus, dyslipidemia, and hypertriglyceridemia [1-3]. From a chemical perspective, it is (2S)-2-Ethoxy.-3-[4-(2-{2-methyl-5-[4-(methylsulfanyl)phenyl]-1H-pyrrol-1-yl}ethoxy)phenyl]Propanoic acid [4]. Saroglitazar has been shown to decrease levels of triglycerides, LDL cholesterol, VLDL cholesterol, and non-HDL cholesterol, while increasing HDL cholesterol, according to clinical research. Additionally, it has demonstrated positive results in glycemic control by decreasing fasting plasma glucose and HBA1c levels in patients with diabetes. Saroglitazar is a pioneering medicine that functions as a dual PPAR agonist, specifically targeting the α (alpha) and γ (gamma) subtypes of the peroxisome proliferator-activated receptor (PPAR). Activation of PPARα reduces elevated levels of triglycerides in the bloodstream, while activation of PPAR γ enhances insulin sensitivity, leading to a decrease in blood sugar levels [5-9].

The literature study identified a limited number of analytical methods that have been described for the quantification of Saroglitazar in pharmaceutical dosage forms. Therefore, the aim of the suggested approach is to create and verify a straightforward, fast, and precise HPLC technique that adheres to ICH requirements. This method will be used to measure the amount of Saroglitazar in both its bulk sample and pharmaceutical formulation.

In the present research work two spectrophotometric methods and one HPLC method have been developed for estimation of saroglitazar from its tablet formulation. The present study was aimed at developing a precise, sensitive, rapid and accurate HPLC method for the analysis of SRG.

Experimental Work

In order to achieve phenomenal retention time and peak asymmetry, a C8 Chromasil column (250 × 4.6 mm, 5 µm

particle size) and to optimize the RP-HPLC parameters, several mobile phase compositions were tried.
A satisfactory separation and good peak symmetry for SRG was obtained with a mobile phase mixture of phosphate buffer (pH 4.5) and methanol at a flow rate of 1 mL/min to get better reproducibility and repeatability. The retention time for SRG was found to be 7.670 min. UV spectra of SRG showed absorbance maxima at 238.2 nm, so this wavelength was selected as the detection wavelength. [10-13] Results from method development and optimization studies are given in Table 1.

Table 1: Optimized Chromatographic Conditions for Estimation of Saroglitazarby HPLC

Parameter	Chromatographic conditions
Statioanry Phase	C8 Chromasil Column (250 × 4.6 mm, 5µm particle size)
Mobile Phase	Mixture of Phosphate Buffer (pH 4.5) and Methanol in Ratio of 25:75, v/v
Flow Rate	1 mL/min
Column Temperature	Ambient temperature
Detection Wavelength	238.2 nm
Injection Volume	20 µl
Run Time	10 min
Retention Time	7.670 min

Method Validation

System Suitability: System suitability test parameters for SRG for the developed method are reported in **Table 2.** % RSD for tailing factor, theoretical plate count, peak area and retention time for SRG were found to be within the limit of 2%, which indicates suitability of the system. The number of theoretical plates and tailing factor were found within the acceptance criteria of >2000 and ≤2.0, respectively, indicating good column efficiency and optimum mobile phase composition.

Table 2: Results from System-suitability Study of Saroglitazarby HPLC

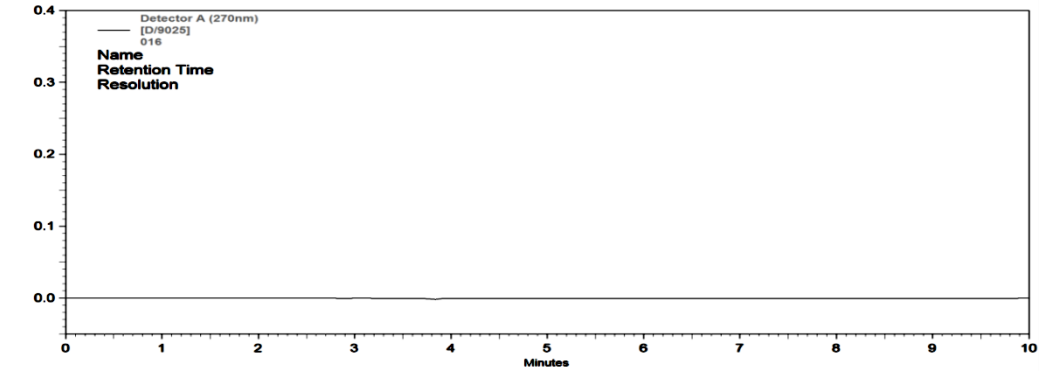
Parameter	SRG (12 µg/mL)	
	Mean (n = 5)	% RSD
Retention time (t_R) min	7.670	0.152
Peak area (A)	795923.2	0.025
Tailing factor (T)	1.301	1.161
No. of theoretical plates (N)	3664.33	1.824

Linearity: The calibration curve for SRG was found to be linear over the range of 4-24 µg/mL (Table 3). The standard chromatogram of SRG is given in Figure 1.

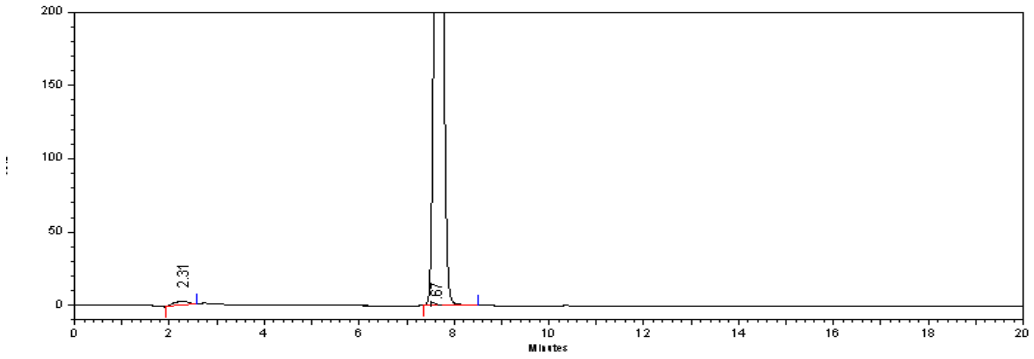
Table 3: Linearity Data of Saroglitazarby HPLC

S. No.	Conc. (µg/ml)	*Peak Area
1.	4	267791
2.	8	524480
3.	12	795518
4.	16	1053228
5.	20	1338071

*Peak area mean of three replicates



(A)



(B)

Figure 1: Chromatograms of (a) Blank (b) Standard Solution of Saroglitazar

After linear regression analysis, the correlation coefficient was found to be 0.999, which shows good correlation between peak area and concentration of drug. The regression graph for SAR is presented in Figure 2. The data of regression analysis is presented in Table 4.

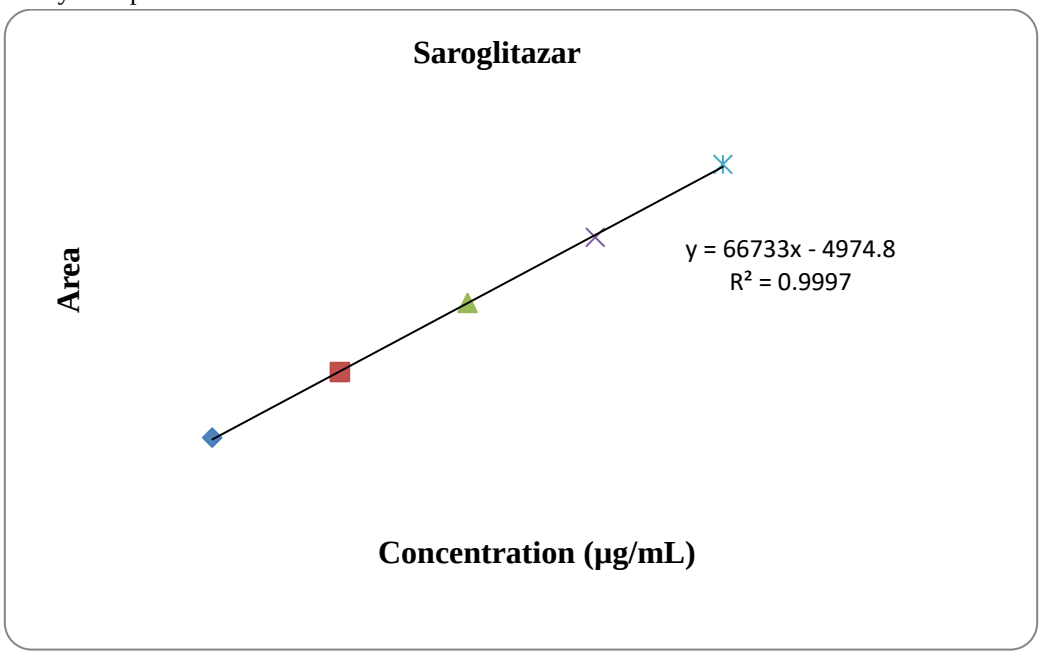


Figure 2: Calibration Curve for Standard Solution of Saroglitazar

Table 4: Results of Regression Analysis of Saroglitazarby HPLC

Parameter	SRG
Linearity range (µg/ml)	4-24
Regression equation (y = mx+c)	y = 66733x – 4974
Slope (m)	66733
Intercept (c)	4974
Correlation coefficient (R^2)	0.999
Limit of detection (µg/ml)	0.418
Limit of quantitation (µg/ml)	1.267

Accuracy/Recovery: The mean recovery of SRG was between 100.34-100.48 % and RSD of recoveries between 0.30-0.43 %, which indicates accuracy of the method **(Table 5).**

Table 5: Accuracy Study for Saroglitazarby HPLC

Accuracy Level (%)	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Mean	SD	% RSD
	6.4	6.45	100.79			
80	6.4	6.42	100.34	100.34	0.45	0.45
	6.4	6.39	99.89			
	8	8.03	100.38			
100	8	8.01	100.15	100.48	0.39	0.39
	8	8.07	100.91			
	9.6	9.62	100.18			
120	9.6	9.64	100.39	100.45	0.30	0.30
	9.6	9.67	100.78			

Precision: The RSD value for SRG was found to be 0.025 **(Table 6)**. The RSDvalue was found to be < 2 %, which indicates that the developed method is repeatable.

Table 6: Repeatability Study for Saroglitazar by HPLC

S. No.	SRG	
	Conc. (µg/ml)	Peak Area
1.		795754
2.		795895
3.	12.0	795747
4.		794986
5.		796234
Avg		**795923.2**
SD		**200.63**
% RSD		**0.025**

The precision studies were performed and the % RSD of the determinations were found to be 0.09-0.13 for intra-day precision and 0.11-0.17 (Table 7) for inter-day precision which are within the limits. Hence the developed method was found to be precise.

Table 7: Precision Study for Saroglitazarby HPLC

Conc.	Intra-day (n=3)		Inter-day (n=3)	
(µg/mL)	Mean ± SD	%RSD	Mean ± SD	%RSD
8	524405±723	0.13	524505±910	0.17
12	796055±992	0.12	796389±1075	0.13
16	1055459±967	0.09	1057321±1192	0.11

Limit of Detection and Limit of Quantitation: Limit of detection was calculated from standard deviation and slope of calibration curve and it was found to be 0.418 µg/ml for SRG.
Limit of quantitation was calculated from standard deviation and slope of calibration curve and it was found to be 1.268 µg/ml for SRG.

Robustness: Two parameters were changed for the study of robustness i.e. composition of mobile phase and flow rate. Small change in these parameters indicated that it did not significantly affect the determination of SRG. Results for robustness is given in table 8.

Table 8: Robustness Study for Saroglitazarby HPLC

S. No	Parameter	Optimized values	Robust conditions	Retention time (t_R), min	Plate Count (N)	Tailing Factor (T)
1	Flow rate	1.0 mL/min	1.1 mL/min	7.594	5852	1.382
			0.9 mL/min	7.766	6535	1.389
2	Mobile phase composition (phosphate buffer : methanol)	250:750	225:775	4.782	6175	1.383
			275:725	5.052	6309	1.386

Acceptance Criteria: Tailing Factor (T) < 2.0, Plate count (N) > 2000, No significant change in Retention time (t_R).

Assay of tablet formulation by HPLC: The developed method was applied to the assay of SRG tablets. From the peak areas the amount of drug present in tablet was estimated. The drug content was calculated as an average of three determinations and assay results were shown in **table 9**. The results were very close to the labeled value of commercial tablets. The representative sample chromatogram of SRG is shown in **Figure 3.**

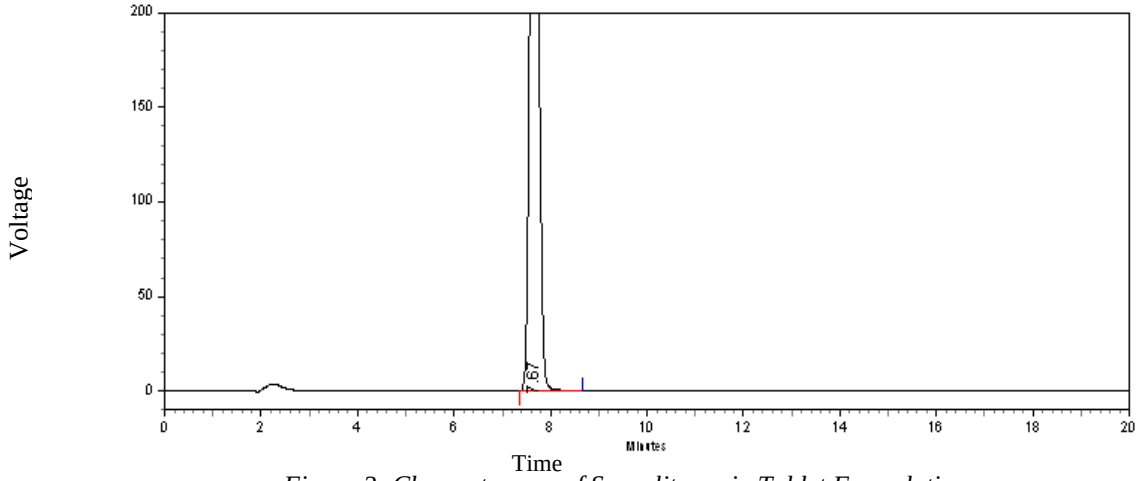


Figure 3: Chromatogram of Saroglitazar in Tablet Formulation

Table 9: Results of Analysis of Tablet Formulation of Saroglitazarby HPLC

Formulation Name	Label Claim (mg)	Amount Found (mg)	% Label Claim
Lipaglyn (Zydus Discovery)	4	3.981	99.69
	4	4.004	100.08
	4	4.029	100.85
Mean		4.005	100.14
SD		0.022	0.582
%RSD		0.581	0.581

Summary and Conclusion

RP-HPLC method was used for the estimation of saroglitazarby using C8 chromasil column. The mobile phase used for the development of method was phosphate buffer (pH 4.5) and methanol in ratio of 25:75, v/v., (pH adjusted 4.5 by ortho-phosphoric acid). Detection was carried out at 238.2 nm. The linearity was observed in the concentration range of 4-20 µg/mL. Run time for the method was 10 min. and the mean retention time for saroglitazar was found to be at 7.670 min at a flow rate of 1.0 ml/min. The result of the analysis by the proposed method is sensitive,

simple, reliable, precise, accurate and robust which indicated good agreement with the label claim of the drug. The method can be used for the routine analysis of the saroglitazar.

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