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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SAROGLITAZAR IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Saroglitazar in tablet dosage form. A C18 Inertsil ODS column (250 × 4.6 mm, 5µm particle size) was used as a stationary phase with a mobile phase containing a mixture of phosphate buffer and acetonitrile in the ratio of 40:60, v/v. The flow rate was 1.0 mL/min. The effluent was monitored at 294 nm and eluted at 2.162 min. Calibration curve was plotted with a range from 20-100 µg/ml for Saroglitazar and the correlation was found to be 0.999. The accuracy range was found between 99.95 -101.07%. The % RSD values for both intraday and interday precision were less than 2.0. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.405µg/ml and 4.260µg/ml respectively. The assay was validated for the parameters like system suitability, precision, accuracy, and robustness parameters. The proposed method can be useful for the routine determination of Saroglitazar in pharmaceutical dosage form.

KEYWORDS: Saroglitazar, Calibration curve, RP-HPLC, Validation, ICH guidelines.

INTRODUCTION

Saroglitazar, chemically, it is (2*S*) - 2- Ethoxy - 3- [4- (2- {2-methyl-5- [4- (methylsulfanyl)phenyl] -1*H*-pyrrol-1-yl} ethoxy)phenyl] propanoic acid **(Fig. 1)**. The chemical formula is $C_{25}H_{29}NO_4S$ and the molecular weight is 439.56 g/mol.[1-3]

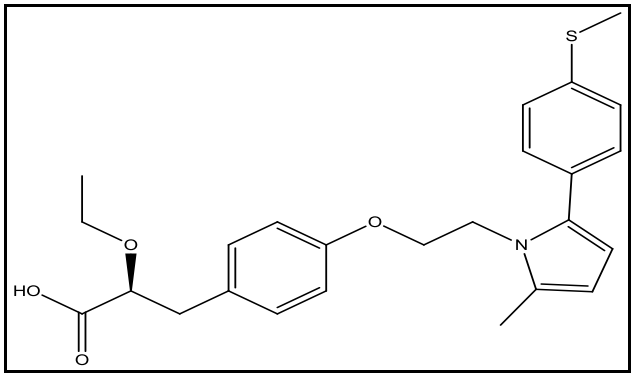

Figure 1: Structure of Saroglitazar.

Saroglitazar is used for the treatment of type 2 diabetes mellitus and dyslipidemia. Saroglitazar is indicated for the treatment of diabetic dyslipidemia and hypertriglyceridemia with type 2 diabetes mellitus not controlled by statin therapy. In clinical studies, saroglitazar has demonstrated reduction of triglycerides (TG), LDL cholesterol, VLDL cholesterol, non-HDL cholesterol and an increase in HDL cholesterol. It has also shown favorable glycemic control by reducing the fasting plasma glucose in diabetes patients. The recommended dose of saroglitazar is one tablet of 4 mg once a day. Saroglitazar is first in this class of drug which acts as a dual PPAR agonist at the subtypes α (alpha) and γ (gamma) of the peroxisome proliferator-activated receptor (PPAR). Agonist action at PPARα lowers high blood triglycerides, and agonist action on PPARγ improves insulin resistance and consequently lowers blood sugar.[4-6]

Literature surveys reveal various methods as UV[7-8], HPTLC[9], HPLC.[10-13] The simple, accurate, precise and validated method for determination of Saroglitazar was developed by UV spectrophotometric method. The developed method was validated as per ICH guidelines.[14]

EXPERIMENTAL

Reagents

The pure drug of saroglitazar was procured as gift sample from Swapnroop Pharmaceutical, Aurangabad, Maharashtra. HPLC grade acetonitrile (ACN), distilled water and analytical grade di-sodium hydrogen phosphate from Merck Pharmaceutical Private Ltd., Mumbai, India were used. Lipaglyn tablets-Zydus Discovery a division of Cadila Healthcare Ltd., Ahmedabad (each tablet contains 4 mg of saroglitazar) were purchased from local market. Membrane filters 0.45 µm and 0.2 µm were procured from Millipore Pvt. Ltd. Bangalore, India.

Instrumentation

LC system used consist of pump (Model Shimadzu; LC-10 AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20µl. Detector consist of photodiode array detector SPD-10 AVP.

Chromatographic conditions

The chromatographic separation was performed using a C18 Inertsil ODS column (250 × 4.6 mm, 5µm particle size). The mobile phase consists of a mixture of phosphate buffer and ACN in ratio of 40:60, v/v. The mobile phase was set at a flow rate of 1 mL/min and the analytes were monitored at 294 nm. The column was maintained at ambient temperature and injected volume was 20 µl. The total runtime was 10 min. The mobile phase was filtered through 0.2 µm membrane filter prior to use. A typical chromatogram of Saroglitazar is shown in **Fig. 2**.

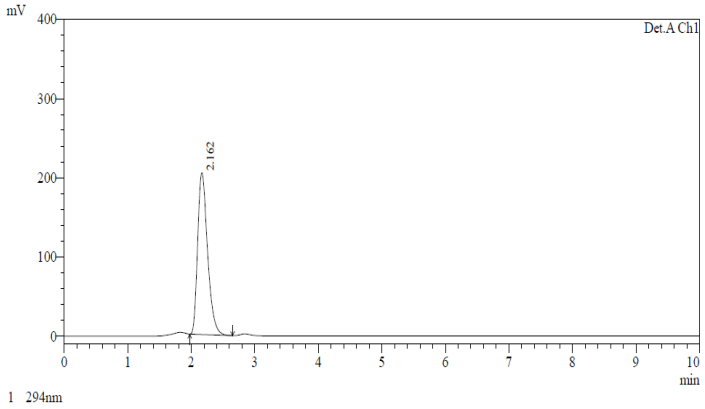

Figure 2: Chromatogram of standard solution of saroglitazar.

Standard Preparation

Accurately weighed 40 mg pure drug of saroglitazar was transferred in a 100 mL clean, dry volumetric flask and mobile phase was added and sonicated to dissolve. The volume was made up to the mark with mobile phase to prepare 400 µg/mL stock solution. 1mL of this solution was transferred into 10mL volumetric flask and volume was made up to the mark with mobile phase to prepare 40 µg/mL standard solution.

Sample Preparation

For the estimation of saroglitazar in the tablet formulation, 20 tablets (label claim 4 mg) were accurately weighed and the average weight per tablet was calculated. The tablets were crushed and finely powdered in glass mortar. Powder equivalent to 4 mg of saroglitazar was accurately weighed and transferred into a 20 mL volumetric flask and

sonicated to dissolve. The volume was made up to the mark with mobile phase, mixed well to prepare 200 µg/mL stock solution. The solution was filtered using 0.2 µm membrane filter and degassed by sonication. 2mL of this solution was transferred into 10mL volumetric flask and volume was made up to the mark with mobile phase to prepare 40 µg/mL test solution. The resulting solution was used as the sample solution for chromatographic analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20µl sample loop of the injection port. The solution was injected five times and the peak areas were recorded.

METHOD VALIDATION

The developed method was validated as per ICH guidelines for its system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) by using the following procedures.

System suitability

System suitability test parameters for saroglitazar for the developed method are reported in **table 1.** % RSD for tailing factor, theoretical plate count, peak area and retention time for saroglitazar 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 1: Results from system-suitability study.

Parameter	saroglitazar (40µg/mL)	
	Mean (n = 5)	%RSD
Retention time (t_R)	2.165	0.060
Peak area (A)	2141101	0.069
Tailing factor (T)	1.462	0.775
No. of theoretical plates (N)	2902.05	0.299

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Saroglitazar at different concentration levels. Peak area of resulting solutions was measured and the calibration curve was plotted between peak area and concentration of the drug (**Fig. 3**). The response was found to be linear in the range 20-100µg/ml for Saroglitazar. The data was given in **table 2, 3**.

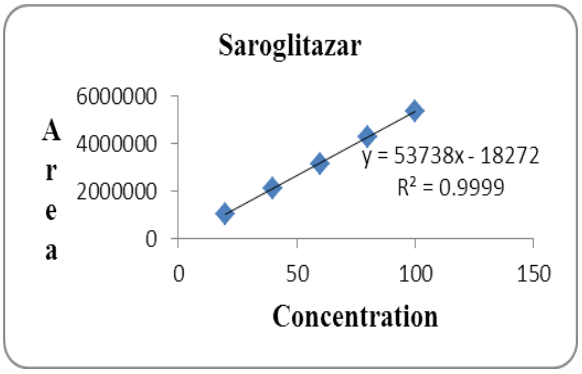

Figure 3: Calibration curve for standard solution of saroglitazar.

Table 2: Linearity data of saroglitazar.

S.No.	Conc. (µg/ml)	* Peak Area
1.	20	1065137
2.	40	2142713
3.	60	3172338
4.	80	4279234
5.	100	5370705

**Peak area mean of three replicates.*

Table 3: Results of regression analysis of saroglitazar.

Parameter	Saroglitazar
Linearity range (µg/ml)	20-100
Regression equation (y = mx+c)	y = 53738x - 18272
Slope (m)	53738
Intercept (c)	18272
Correlation coefficient (R^2)	0.999
Limit of detection (µg/ml)	1.405
Limit of quantitation (µg/ml)	4.260

Accuracy

Accuracy was performed in triplicate for various concentrations of Saroglitazar equivalent to 80%, 100% and 120% of the active ingredient, by adding a known amount of saroglitazar standard to a fixed amount of the preanalysed sample of saroglitazar. The recovered amount of saroglitazar, % recovery and %RSD of each level was calculated. The data was given in **table 4**.

Table 4: Accuracy data for saroglitazar.

Accuracy Level (%)	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Mean	SD	%RSD
80	32	32.09	100.29	101.07	0.71	0.71
	32	32.38	101.21			
	32	32.54	101.71			
100	40	39.99	99.98	99.95	0.27	0.27
	40	39.86	99.66			
	40	40.08	100.21			
120	48	47.66	99.29	100.05	0.68	0.68
	48	48.11	100.23			
	48	48.30	100.63			

Precision

Repeatability

Five sample solutions of the same concentration were prepared and injected into the HPLC system as per test procedure. The results were given in **table 5**.

Table 5: Repeatabilty study for saroglitazar.

S.No.	Saroglitazar	
	Conc. (µg/ml)	Peak Area
1.	40.0	2140031
2.		2139388
3.		2140939
4.		2142059
5.		2143088
Avg		2141101
SD		1497.61
% RSD		0.069

Precision (Day to Day variability)

Intra-day precision was investigated by replicate applications and measurements of peak area for saroglitazar for three times on the same day under similar conditions. Inter-day precision was obtained by repeating the assay three times on different days. The percent relative standard deviation (% RSD) was calculated. The results were given in **table 6**.

Table 6: Precision study for saroglitazar.

Conc. (µg /mL)	Intra-day (n=3)		Inter-day (n=3)	
	Mean ± SD	%RSD	Mean ± SD	%RSD
20	1065172± 1352	0.12	1065322±1751	0.16
40	2227260±5401	0.24	2229673±6969	0.31
60	3025804±3237	0.10	3027754±3689	0.12

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines.

Robustness

Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provide an indication of its reliability during normal usage. Robustness can be determined by analysis of solution by changing physical parameters like composition of mobile phase and flow rate, In order to measure the extent of method robustness, the parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The results by small variations in these parameters as shown in **table 7**.

Table 7: Robustness study for saroglitazar

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 0.9 mL/min	1.925 2.384	2771 2749	1.342 1.387
2	Mobile phase composition (Phosphate Buffer:ACN)	40:60	35:65 45:55	1.946 2.381	2815 2749	1.462 1.256

Acceptance criteria: *Tailing Factor (T) < 2.0, Plate count (N) > 2000, significant change in Retention time (R_t).*

Assay

The developed method was applied to the assay of saroglitazar 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 8**. The results were very close to the labeled value of commercial tablets. The representative chromatogram of saroglitazar is shown in **Fig. 4**.

Table 8: Results of analysis of formulation

Formulation name	Label Claim (mg)	Amount found (mg)	% Label claim
Lipaglyn (Zydus Discovery)	4	3.983	99.59
	4	4.003	100.08
	4	4.030	100.75
Mean		4.005	100.14
SD		0.023	0.581
%RSD		0.580	0.580

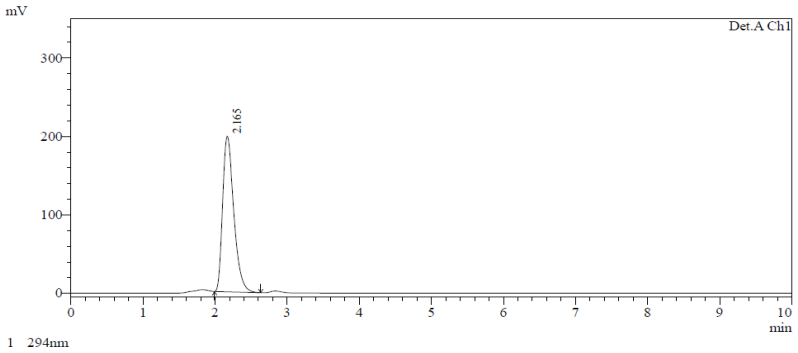

Figure 4: Chromatogram of saroglitazar in tablet formulation.

RESULTS AND DISCUSSION

A reverse-phase HPLC method was proposed as a suitable method for the determination of Saroglitazar in tablet dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, phosphate buffer and acetonitrile in the ratio 40:60 v/v was used as mobile phase, which showed good resolution of Saroglitazar peak. The wavelength of detection selected was 294 nm, as the drug showed sharp and better peak shape at this wavelength. By developed method the retention time of Saroglitazar was about 2.162 min. The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the developed method was suitable for determination of drug in pharmaceutical formulation has no interference of additives. Hence the developed method can be applied for estimation of saroglitazar in marketed formulation.

CONCLUSION

The developed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of saroglitazar in tablet dosage form. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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