# Method Development and Validation for the Estimation of Saroglitazar in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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**ABSTRACT**

*A simple, rapid, precise and accurate RP-HPLC method was developed and validated for the determination of Saroglitazar in tablet dosage form. Separation of the drug was achieved on a Kromasil C18 Column (150 mm x 4.6 mm I.D., 5 µm particle size). The method showed a linear response in the concentration range of 10-60 µg/ml using 0.1% orthophosphoric acid buffer:acetonitrile as the mobile phase in the ratio of 45:55 v/v with detection at 295 nm with a flow rate of 1 ml/min and retention time was 3.430 min. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of Saroglitazar in tablet dosage form.*

**Keywords**: *Saroglitazar, estimation, UV detection, dosage form.*

## INTRODUCTION

Saroglitazar (Figure 1) is a drug for the treatment of type 2 diabetes mellitus, dyslipidemia and hyper triglyceridemia[1-3]. Chemically, it is (2*S*)-2-Ethoxy-3-[4-(2-{2-methyl-5-[4-(methyl sulfanyl)phenyl]-1*H*-pyrrol-1-yl}ethoxy)phenyl] propanoic acid[4]. In clinical studies, Saroglitazar has demonstrated reduction of triglycerides, LDL cholesterol, VLDL cholesterol, non-HDL cholesterol and an increase in HDL cholesterol[5]. It has also shown favorable glycemic control by reducing the fasting plasma glucose and $HBA_{1c}$ in diabetes patients. Saroglitazar is novel first in class drug which acts as a dual PPAR agonist at the subtypes α (alpha) and γ (gamma) of the peroxisome proliferator-activated receptor (PPAR)[6]. Agonist action at PPARα lowers high blood triglycerides, and agonist action on PPAR γ improves insulin resistance and consequently lowers blood sugar[7].

Literature survey revealed that few analytical methods such as UV[8,9], HPLC[10,11] and HPTLC[12] methods were reported for the determination of Saroglitazar in pharmaceutical dosage form. Hence the objective of the proposed method is to develop and validate a simple, rapid and accurate HPLC method in accordance with ICH guidelines[13] for the determination of Saroglitazar in bulk sample and its pharmaceutical formulation.

## MATERIALS AND METHODS

***Chemicals and reagents:*** The standard bulk drug sample of Saroglitazar was provided as gift sample from Spectrum Pharma Research Solutions, Hyderabad, India. The market formulation LIPAGLYN tablets (Saroglitazar 4 mg) were procured from local market. HPLC grade acetonitrile and water were purchased from E.Merck (India) Ltd, Mumbai, India. Orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

***Instrumentation:*** The analysis of drug Saroglitazar was carried out on a Waters HPLC system equipped with a reverse phase Kromasil C18 Column (150 mm x 4.6 mm I.D., 5 µm particle size), a 2695 binary pump, a 20 µl injection loop, auto sampler, a 2487 dual absorbance detector and running on Waters Empower 2 software.

***Chromatographic conditions:*** A mixture of 0.1% orthophosphoric acid buffer and acetonitrile as the mobile phase in the ratio of 45:55 v/v was found to be the most suitable mobile phase for ideal chromatographic separation of Saroglitazar. The solvent mixture was filtered through 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. Injection volume was 10 µl and the column was maintained at a temperature of $30^0$C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. Water and acetonitrile was used as diluent in the ratio of 50:50 v/v. The detection of the drug was monitored at 295 nm. The run time was set at 6 minutes.

***Preparation of stock and working standard solutions:*** 4 mg of Saroglitazar was accurately weighed, transferred to 10 ml volumetric flask and is dissolved in 7 ml of the diluent. Sonicate the solution for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume is made up to the mark with diluent to get a stock solution. Further pipetted 1.0 ml of the above stock solution into a 10 ml volumetric flask and

diluted up to the mark with diluent to obtain required working standard concentrations of 10-60 µg/ml.

***Preparation of sample solution:*** Twenty commercial tablets of Saroglitazar were weighed and powdered. A quantity of the powder equivalent to 4 mg of Saroglitazar was accurately weighed, transferred to 10 ml volumetric flask and is dissolved in 7 ml of the diluent. Sonicate the solution for few minutes to dissolve the drug completely. Then it was filtered through 0.45 µ filter and the volume is made up to the mark with diluent. Further pipetted 1.0 ml of the above solution into a 10 ml volumetric flask and diluted up to the mark with diluent to obtain required concentrations of Saroglitazar in pharmaceutical dosage form. Inject 10 µl of the above solutions into the HPLC system and the chromatograms were recorded. All experiments were conducted in triplicate.

## METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, system suitability and specificity. Standard plots were constructed with six concentrations in the range of 10-60 µg/ml prepared in triplicates to test linearity. The peak area of Saroglitazar was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both system precision and method precision. The precision was calculated from six replicate injections of freshly prepared Saroglitazar test solution in the concentration value of 100% (40 µg/ml) of the intended test concentration value. Peak area of the Saroglitazar was determined and precision was reported as %RSD. Method accuracy was tested (% recovery of individual measurements) by analyzing sample of Saroglitazar at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Saroglitazar recovered in the samples.

***Linearity:*** Several aliquots of standard solution of Saroglitazar was taken in different 10 ml volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Saroglitazar were in the linearity range of 10-60 µg/ml. Evaluation of the drug was performed with UV detector at 295 nm, peak area was recorded for all the peaks. The linearity results were shown in Table 1 and the calibration curve of Saroglitazar was shown in Figure 2.

***Precision:*** Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as system precision and method precision. To study the system precision, six replicate standard solutions of Saroglitazar was injected. The percent relative standard deviation (%RSD) was calculated. To study the method precision, six preparations from the same samples of Saroglitazar was injected and the %RSD were calculated. Results of precision studies were shown in Table 2.

***Accuracy:*** The accuracy of the method was assessed by recovery study of Saroglitazar in the dosage form at three different concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. At each level, three determinations were performed. The concentration of Saroglitazar was calculated at different levels. The accuracy results were shown in Table 3.

***Limit of detection and limit of quantification:*** The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method.

***Ruggedness and robustness:*** The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Robustness of the method was determined by making slight changes in the chromatographic conditions such as the composition of the mobile phase and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated.

***System suitability:*** System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

***Specificity:*** The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample). Saroglitazar drug solution of 40 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of from any of the impurities, if present.

***Analysis of Saroglitazar in tablet dosage form:*** 10 µl of Saroglitazar sample solution was injected and from the peak area of Saroglitazar, amount of drug in samples were computed. The assay results are furnished in Table 4.

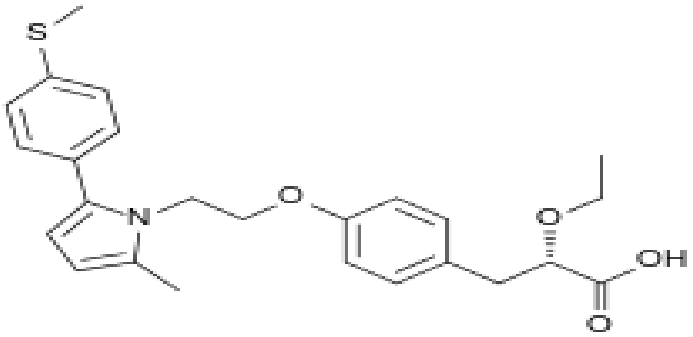


**Fig. 1: Chemical structure of Saroglitazar**

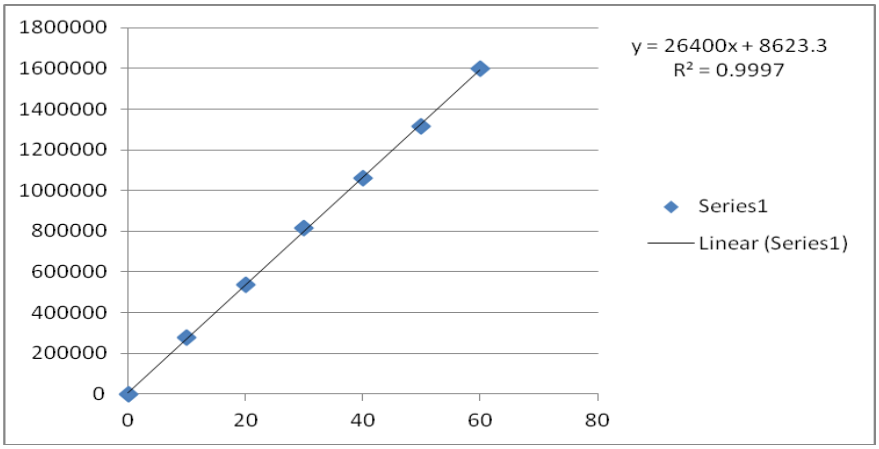


**Fig. 2: Calibration curve of Saroglitazar**

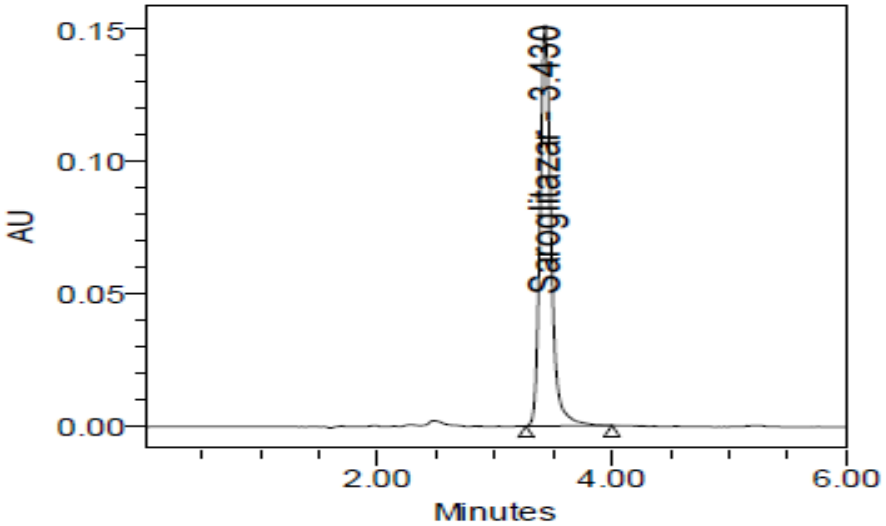


**Fig. 3: Typical chromatogram of Saroglitazar standard**

**Table 1: Linearity of Saroglitazar**

| Linearity level | Concentration (µg/ml) | Area |
|---|---|---|
| I | 10 | 277874 |
| II | 20 | 538549 |
| III | 30 | 813776 |
| IV | 40 | 1060831 |
| V | 50 | 1314584 |
| VI | 60 | 1598781 |

**Table 2: Precision studies of Saroglitazar**

| Injection number | System precision area | Method precision area |
|---|---|---|
| 1 | 958352 | 984075 |
| 2 | 956768 | 989787 |
| 3 | 964601 | 980649 |
| 4 | 960517 | 988127 |
| 5 | 957809 | 990253 |
| 6 | 953188 | 985748 |
| Mean | 958539 | 986440 |
| SD | 3820.9 | 3693.1 |
| % RSD | 0.4 | 0.4 |

**Table 3: Recovery studies of Saroglitzar**

| % Concentration (at specification level) | Concentration added (µg/ml) | Concentration found (µg/ml) | % Recovery | Mean recovery |
|---|---|---|---|---|
| 50% | 20 | 19.97 | 99.85% | 100.01% |
| 100% | 40 | 40.16 | 100.40% | |
| 150% | 60 | 59.88 | 99.80% | |

**Table 4: Analysis of Saroglitazar formulation**

| Formulation | Label claim | Amount found | % Assay |
|---|---|---|---|
| Lipaglyn | 4 mg | 3.99 mg | 99.75% |

**Table 5: Chromatographic conditions of Saroglitazar**

| Parameter | Condition |
|---|---|
| Mobile Phase | Buffer:acetonitrile (55:45% v/v) |
| Diluent | Water:acetonitrile(50:50% v/v) |
| UV detection | 295 nm |
| Analytical column | Kromasil C18 column (150 x 4.6 mm, 5µ) |
| Flow rate | 1.0 ml/min |
| Temperature | $30^0$C |
| Injection volume | 10 µl |
| Run time | 6.0 min |

**Table 6: System suitability parameters of Saroglitazar**

| Parameter | Results |
|---|---|
| λmax (nm) | 295 |
| Linearity range (µg/ml) | 10-60 |
| Correlation coefficient | 0.9997 |
| Retention time (min.) | 3.430 |
| Theoretical plates (N) | 7073 |
| Tailing factor | 1.23 |
| Limit of detection (µg/ml) | 0.143 |
| Limit of quantification (µg/ml) | 0.434 |

## RESULTS AND DISCUSSION

The method was optimized with a view to develop an precise and accurate RP-HPLC method for determination of Saroglitazar in tablet dosage form using Kromasil C18 Column (150 mm x 4.6 mm I.D., 5 µm particle size) in isocratic mode with mobile phase composition of 0.1% orthophosphoric acid buffer and acetonitrile as the mobile phase in the ratio of 45:55 v/v. The use of above mobile phase resulted in peak with good shape and resolution. The flow rate was 1.0 ml/min and the drug component was measured with UV detector at 295 nm. The results of optimized chromatographic conditions were shown in Table 5. The response for the drug Saroglitazar was linear in the concentration range of 10-60 µg/ml. The regression equation was found to be y=26400x+8623.3 and correlation coefficient value of Saroglitazar was found to be 0.9997. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The %RSD for system precision and method precision for Saroglitazar were found to be 0.4% and 0.4%, which are well within the acceptable criteria of not more than 2.0 and indicate the method is precise. The mean recovery of Saroglitazar was found to be 100.01%

that shows there is no interference from excipients and indicate the method is accurate. The limit of detection and limit of quantification for Saroglitazar were found to be 0.143 µg/ml and 0.434 µg/ml, which indicate the sensitivity of the method. It was observed that there were no significant changes in the chromatograms, which demonstrated that the HPLC method so developed is rugged and robust.

The retention time of Saroglitazar was 3.430 min. The number of theoretical plates was 7073 and tailing factor was 1.23 for Saroglitazar, which indicates efficient performance of the column. The summary of system suitability parameters were shown in Table 6. Typical chromatogram of drug Saroglitazar standard was shown in Figure 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed HPLC method and hence the method was found to be specific and also confirmed with the results of analysis of formulation. Validated method was applied for the determination of Saroglitazar in commercial formulations. The % assay of Saroglitazar was found to be 99.75% of label claim of Saroglitazar.

## CONCLUSION

A validated RP-HPLC method has been developed for the determination of Saroglitazar in tablet dosage form. The proposed method showed acceptable accuracy, precision, selectivity and wide linear concentration range. The results of analysis proved that the method is suitable for the determination of Saroglitazar in bulk and tablet dosage form and it is recommended for routine quality control analysis of the Saroglitazar in pharmaceutical formulation.

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