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DEVELOPMENT OF VALIDATED RP-HPLC METHOD FOR ASSAY AND DISSOLUTION ANALYSIS OF SAROGLITAZAR

Atul T. Hemke*, Balram M. Jadhao, Suraj B. Ghorpade, Milind J. Umekar

Department of Pharmaceutical Chemistry,
Smt. KishoritaiBhoyar College of Pharmacy, Kamptee, Nagpur, Maharashtra - 441002
*Corresponding author E-mail: atulhemke321@gmail.com

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ABSTRACT

The proposed work represents development of an isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method for the determination of Saroglitazar in bulk and for pharmaceutical dosage form. The Shimadzu HPLC system equipped with UV visible detector, Phenomenox ODS C18 (250 mm x 3.9 mm; 5µ) column and separation was achieved using mobile phase composed of mixture of disodium hydrogen phosphate buffer and acetonitrile in a ratio of 42:58 v/v at a flow rate of 1 ml/min. The wavelength 294nm was selected as detection wavelength during experimentation. The optimized dissolution parameters includes mainly phosphate buffer (pH-6.8) as dissolution media and type II paddle apparatus. The release was found maximum with agitation speed of 50rpm as compared to 75rpm. The % estimation of Saroglitazar by the developed method was found satisfactory shows mean value 98.44%.The linearity of the detector response test method indicates coefficient of correlation value 0.998.The proposed method was validated as per ICH guidelines and the results were found within limit of acceptance. The proposed method can be adopted for the routine analysis of Saroglitazar in any quality control laboratory.

INTRODUCTION

Saroglitazar (Lipaglyn) is indicated for the treatment of diabetic dyslipidemia and hypertriglyceridemia with type 2 diabetes mellitus not controlled by statin therapy [1]. In clinical studies[2-4] Saroglitazar has demonstrated reduction of triglycerides (TG), LDL cholesterol, VLDL cholesterol, non-HDL cholesterol and an increase in HDL cholesterol a characteristic hallmark of atherogenic diabetic dyslipidemia (ADD). Chemically Saroglitazar is (2S)-2-Ethoxy-3-[4-(2- {2-methyl-5- [4- (methylsulfanyl) phenyl]-1H-pyrrol-1-yl} ethoxy)phenyl] propanoic acid with molecular formula $C_{25}H_{29}NO_4$Sand molecular weight 439.56. The dissolution can be defined in a narrow sense as the process by which a solid substance is incorporated into the solvent to form a solution. However, in a broad sense, it is more than a simple measurement of solubility rate and can be better described as physical test to predict the drug release from a dosage form, for a given area for some precise time. Fundamentally, this process is controlled by the affinity between the solvent and the solid substance and the way by which the pharmaceutical system releases the drug[1-2]. According to Mehta and coworkers[3]dissolution test provides an indication of bioavailability of a drug and thus, pharmaceutical equivalence from batch to batch. The dissolution test is an important tool in quality control of drugs and it becomes more important for drugs[9-12]. An extensive literature survey was carried out and found some analytical methods were reported for estimation of said drug in bulk as well in pharmaceutical dosage form[14-16]. The present investigation describes development of rapid, economical and precise RP-HPLC method for estimation of drug followed by validation. Also attempts

were made to develop dissolution test method for Saroglitazar as dissolution testing has emerged in the pharmaceutical field as a very important tool to characterize drug performance.

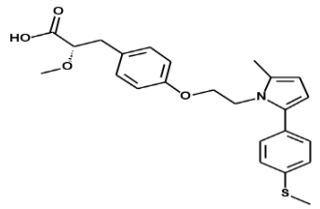

Figure 1: Chemical Structure of Saroglitazar

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade Saroglitazar standard was obtained as generous gift from Cadila healthcare limited, Ahmedabad, India.The commercially available formulation of Saroglitazarwas purchased from local market.HPLC grade Acetonitrilewas purchased from Himedia Co; Mumbai, India and Ortho Phosphoric acid, Hydrochloric acid, Sodium Hydroxide and Hydrogen Peroxide, Disodium Hydrogen Phosphate of AR grade were purchased from LobaChem Mumbai, India.

Instrumentation

Dissolution Apparatus: Electrolab, Tablet Dissolution tester: TDT 06P, Lab India Ds1400, HPLC: Shimadzu HPLC Analyitical P-300 series, UV-Spectrophotometer: Shimadzu UV-1700 double beam, Sonicator: PCI Mumbai 3.5L 100H, Spectra lab. UCB-300, pH-meter: Global Model No. DPH-500; EI Model No. 1102012 and Digital Balance: Shimadzu AUX220. The spinchrome software was used throughout the experimentation for acquisition of chromatographic data.

Methodology

The column was equilibrated with the mobile phase for at least 30 minutes prior to the injection of the standard or sample solution. The working standard solution of concentration 40µg/mL was injected into the column; the elution was carried out with mobile phase at constant flow rate, and the eluted components were detected at a suitable wavelength using UV Visible detector. The optimization of the proposed RP-HPLC method was carried out by varying one of the chromatographic

Development of RP- HPLC method

Preparation of standard solution

An accurately weighed quantity about 10mg of Saroglitazar was transferred to 10.0ml volumetric flask and volume was made up to the mark with diluent (S). A 5.0 ml of solution (S) was transferred into 50.0ml volumetric flask, added about 35.0ml of diluent, sonicate to dissolved and diluted up to the mark with diluent (S_1). A 4.0ml of solution (S_1) further diluted to 10.0 ml with diluent to get concentration 40µg/ml (S_2). The diluent was prepared by mixing buffer and acetonitrile.

Selection of wavelength

The working standard solution of Saroglitazar (40µg/ml) was scanned in the range of 400-200nm in 1.0cm cell against solvent blank (diluent) and the spectra was recorded as shown in Figure 2.From the recorded UV spectra, Saroglitazar shows maximum absorbance at 294nm and selected as detection wavelength.

Optimization of mobile phase

Preparation of mobile phase

Buffer solution of disodium hydrogen phosphate (DSHP) was prepared by dissolving an accurately weighed amount of 1.41gm of (DSHP) in 1lit of HPLC double distilled water, and pH of the solution was adjusted to 7.0 with dilute Ortho Phosphoric acid. The solution was filtered through 0.45µm membrane filter and sonicated by using Power Sonicator. Polar nature diluent was prepared by mixing Buffer solution and Acetonitrile in the ratio 42:58 %v/v which was used in the preparation and dilution of standard and sample solutions.

conditions such as polarity of the column, composition of mobile phase, injection volume, flow rates and detection wavelength keeping other constant and chromatographic parameters such as retention time, number of theoretical plates, tailing factor, area of the peak and peak height were obtained. Finally, About 20µl of working standard solution of concentration 40µg/ml injected into Phenomenox ODS C18 (250 mm x 4.6 mm; 5µ) column at ambient temperature and a mobile phase of mixture of Disodium Hydrogen Phosphate Buffer and Acetonitrile in a ratio of 42:58 %v/v at a flow rate of 1 ml/min and wave length was detected at 294 nm using UV-Visible detector for the separation. The optimized chromatographic parameters are shown in Table 1.

Study of system suitability parameters

The chromatographic conditions were set as per the optimized parameters and the mobile phase was allowed to equilibrate to get steady base-line. The study of the system suitability parameters were carried out using six replicate injection of standard solution (20µl) was injected and chromatogram recorded.

Preparation of sample solution for assay

Weighed and crushed powder of 20 intact tablets transferred in 100.0 ml volumetric flask. To it 70.0 mL of mobile phase was added and sonicated for 15 minutes and dilutedupto mark. The contents were allowed to stand for 10 minutes and 4.0 ml of supernatant solution was further diluted to 25.0 mL with mobile phase. The prepared sample solution was filtered through 0.45µ nylon membrane filters.

Solubility study for saroglitazar

Solubility study of saroglitazar was carried out by dissolving the drug in different dissolution media so as to obtain the saturated solution. If clear solution is obtained, further 10.0 mg of drug was added to dissolution media and the procedure was repeated until the saturated solution was obtained. The point, at which the saturated solution was obtained, used to determine the saturated solubility in per 250.0 ml of respective dissolution media.

Preparation of test solution

Weighed and drop one tablet in each of six dissolution vessels containing the dissolution media for the drug under analysis. After specified time point 10.0 mL of the aliquot was withdrawn. Six injections of standard solution were injected in to chromatographic system followed by the test sample collected at each time interval. After each sixth injection the bracketing standard solution were injected to assure the suitability of system. Using optimized chromatographic conditions, the amount of drug released at each time interval was estimated.

Trials for selection of dissolution media:

Various dissolution profiles were carried out in different media depending on solubility and drug release. The various trials were taken using different dissolution medium are as follows:

Dissolution parameters:

Apparatus : USP type II/I
Speed : 50/75 rpm
Temperature : 37°C (±5°C)
Volume : 500/900ml
Profile time points: 5, 10, 15, 20,30,45,60 minutes

Dissolution method validation

As per ICH guidelines, validation of proposed method was carried out.

RESULTS AND DISCUSSION

The chromatographic conditions were set as per the optimized parameters; mobile phase was allowed to equilibrate with stationary phase as indicated by steady baseline. A 20µl of solution was injected through manual injector and chromatogram was recorded. A mobile phase containing mixture of Disodium Hydrogen Phosphate Buffer and Acetonitrile in a ratio of 42:58 %v/v gave well-resolved peak and reasonable retention time as shown in Figure 3.

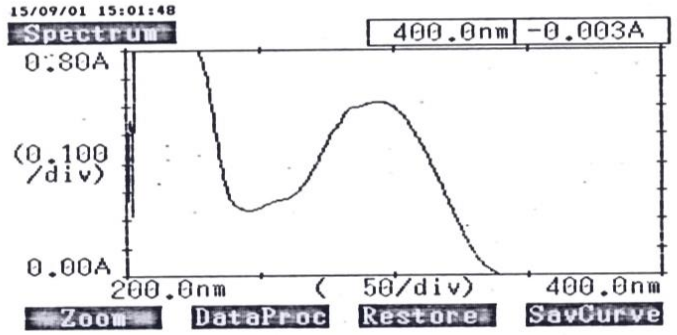

Figure 2: UV Spectrum of standard Saroglitazar

Table 1: Optimized chromatographic parameters

Parameter	Condition
Detection wavelength	294nm
Column	Phenomenox ODS C18 (250 mm x 4.6 mm; 5µ)
Flow rate	1 ml/min
Column temperature	45°C
Injection volume	20 µL
Run time	10 min
Mobile phase	Buffer: Acetonitrile (42:58 %v/v)

System suitability parameters: After equilibration of column with mobile phase, six replicate injections of 20µl solution were injected through the manual injection and the chromatograms were recorded and the peak area was noted. The recorded results of system suitability parameters are shown in Table 2.

Estimation of saroglitazar: The content of saroglitazar in each sample was calculated by comparing the peak area of sample with that of standard, the results obtained and statistical data are shown in Table 3.

Dissolution study: Various dissolution profiles were carried out in different media depending on solubility and drug release. The various trials were taken using different dissolution medium and finally Phosphate buffer (pH 6.8) selected as dissolution medium. The obtained chromatograms are shown in figure 5a-5d.

Apparatus : USP type II
Speed : 50 rpm
Temperature : 37°C (±5°C)
Medium : Phosphate buffer (pH 6.8) (900.0 mL)
Profile time points: 5, 10, 15, 20, 30, 45, 60 minutes and recovery

Calculation of percent release of saroglitazar

The % drug release of saroglitazar in selected dissolution media (Phosphate buffer (pH 6.8)) was calculated using the following formula:

$$\% \text{ Release} = \frac{\text{vol.of disso medium}}{\text{LC}} \times \frac{\text{AT}}{\text{AS}} \times \text{con.(ws)} \times 100$$

Where,AT: Peak area of saroglitazar in the chromatogram of sample

AS: Average peak area of saroglitazar in the chromatogram of standard solution

WS: Weight of saroglitazar working standard in mg
LC: Labeled claim in mg

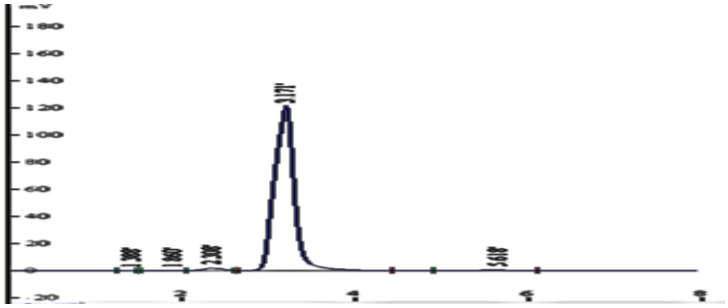

Figure 3: Chromatogram of standard Saroglitazar

Table 2: Results for system suitability parameters

Sr. No.	Standard weight taken in (mg)	Area(mV)
1	10.0	964.07
2		956.03
3		941.50
4		966.81
5		958.07
6		975.56
	Mean	960.34
	± SD	11.54
	%RSD	1.20
	Retention time	3.177
	Tailing factor(Asymmetry)	0.186
	Theoretical plate	2759

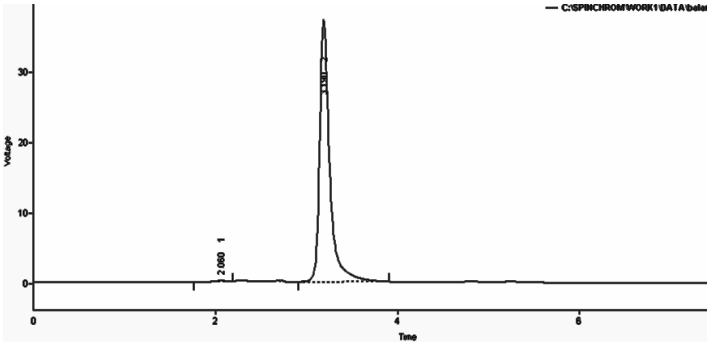

Figure 4(a): Chromatograph for standard Saroglitazar

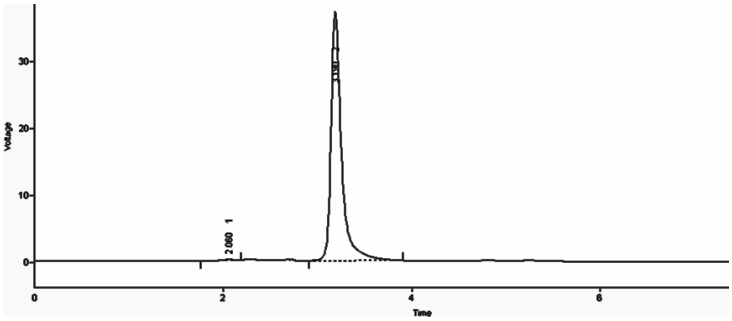

Figure 4(b): Chromatograph for sample Saroglitazar

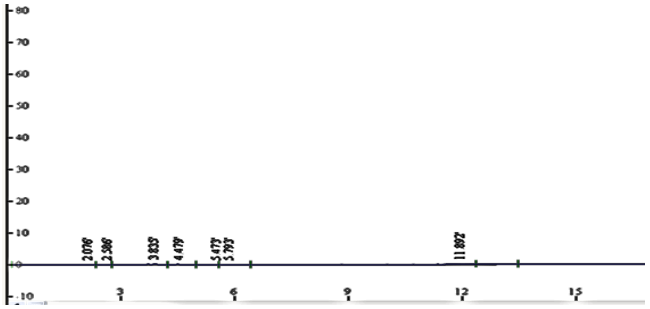

Figure5(a):A Chromatogram for blank

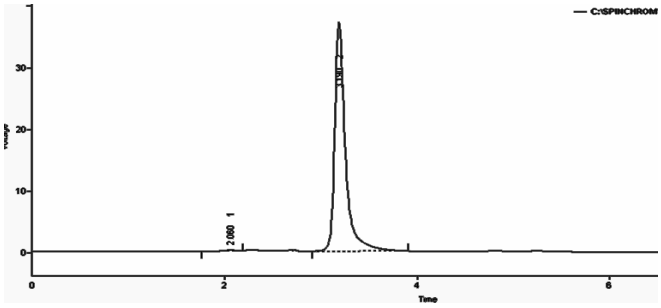

Figure5(b): A Chromatogram for standard drug solution

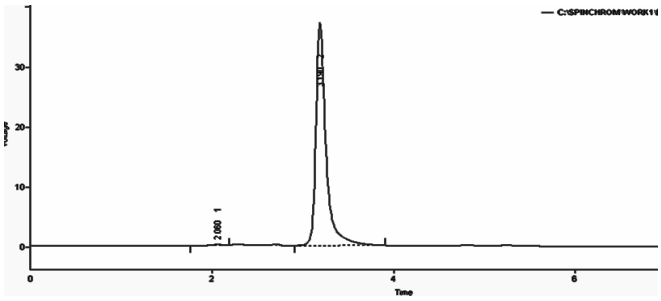

Figure5(c): A Chromatogram for sample at time 5 min

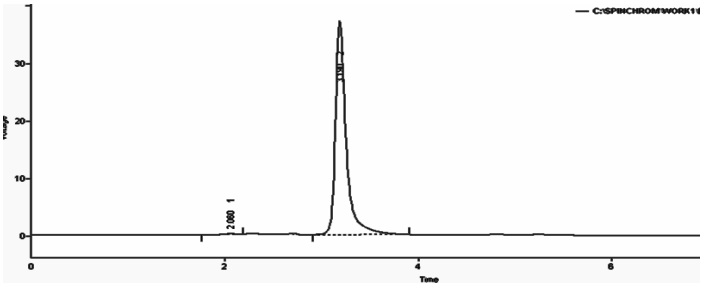

Figure 5 (d): A Chromatogram for sample at time infinity

Optimized dissolution parameters: It was observed that the release of drug in USP TYPE –I (Basket) apparatus was fast as compared to USP TYPE-II (Paddle) apparatus. Using type-II apparatus proper profiling for drug release was observed. Therefore it was selected as one of the optimized dissolution parameter. Also the release of drug at first time point was maximum when the speed of rotation was changed from 50 rpm to 75 rpm i.e. very fast release was observed at 75 RPM as compared to 50 rpm using USP TYPE- II apparatus.From the above all, finalized dissolution parameters for the analysis of saroglitazar are as follows

Dissolution method validation: As per ICH guideline, validation of proposed method was carried out.

Linearity of test method: The linearity of test method is (within a given range) to obtain test result which are directly proportional to the concentration of analyte in the sample. The linearity of detector response for drug with respect to concentration was demonstrated by considering label claim of respective drugs under analysis as 100% target concentration and preparing five solutions in the diluent with concentration ranging from 10% to 150% of the target concentration. The linearity of the detector response test method was established by plotting a graph between mean peak areas verses concentration of drug and determined the correlation coefficient ($R^2 = 0.9983$).

Accuracy of test method: The accuracy of the test method expresses the closeness of the agreement between the value which is accepted either as a conventional true value or an accepted reference value.

Procedure: An accurately weighed six saroglitazar tablets were drop into the dissolution vessels after some time standard drug was added to it at three different concentration levels. After 45 min, samples were withdrawn from dissolution vessel and 20µl of each solution injected to chromatographic system. From the recorded chromatogram, peak area was calculated. The observation and results are tabulated in Table 6.

Precision: The test solution was obtained by performing the dissolution of the drug under optimized dissolution parameters. The system was allowed to equilibrate for 30 min by passing the mobile phase through the chromatographic column at the rate of 1ml/min. After equilibration, five injections of standard Saroglitazar of 20µl were injected to check the system suitability followed by the injection of test solution. The observation and results of precision study are tabulated in Table 7.

Robustness of test method: The robustness of analytical method is the measure analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in parameters internal to the procedure. The robustness of the method was evaluated by injecting the sample at deliberately varied the chromatographic condition i.e. detection wavelength (±5 nm), column temperature ($\pm 5^0$C), change into flow rate (±2 ml/min.) and the system suitability parameter was evaluated. The observation and results of robustness study are tabulated in Table 8(a-c).

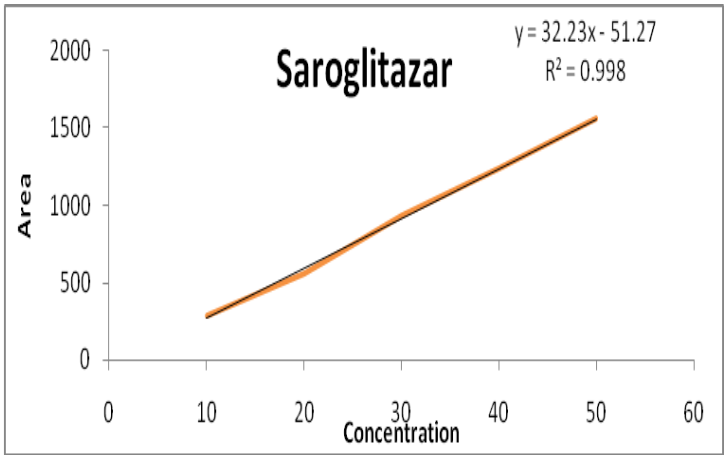

Figure 6: Linearity plot of Saroglitazar

Table 4: Observations of saroglitazar analysis

Time point (min)	Sample	Retention time	Area (mV)	Asymmetry	Theoretical plates	% Release
	Standard	3.30	953	1.73	3768	-
5	Test	3.66	140.74	1.17	4182	73.84
10		3.63	150.77	1.19	4072	79.10
15		3.34	155.54	1.14	3724	81.60
20		3.24	174.03	1.11	5340	91.30
30		3.33	178.53	1.07	3197	93.66
45		3.32	185.61	1.18	2893	97.38
60		3.16	190.22	1.06	3817	99.68
Recovery		3.25	193.06	1.17	2856	101.25

Table 5: Representing the optimized dissolution parameters

Drug	Dissolution media	Media volume	USP Apparatus	Speed of rotation
Saroglitazar	Phosphate buffer (pH 6.8)	500 mL	Type II Paddle	50 rpm

Table 6: Observations and results of accuracy study

Accuracy level	Amount of drug added (in mg)	Peak Area (mV)	Total amount estimated (mg)	% Recovery
50%	2.03	285.33	6.00	100.07
	2.05	285.76	6.12	102.91
100%	4.02	380.66	8.22	103.90
	4.01	381.54	7.98	99.01
150%	6.02	570.66	10.11	101.37
	6.04	570.78	10.08	100.74
			Mean	101.32
			±SD	1.66
			%RSD	1.64

Robustness of test method: The robustness of analytical method is the measure analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in parameters internal to the procedure. The robustness of the method was evaluated by injecting the sample at deliberately varied the chromatographic condition i.e. detection wavelength (±5 nm), column temperature (±5^{0}C), change into flow rate (±2 ml/min.) and the system suitability parameter was evaluated. The observation and results of robustness study are tabulated in Table 8(a-c).

Table 7: Observations and results of precision study

Preparation No.	% Release of Saroglitazar at 45 min
1	96.74
2	97.42
3	97.63
4	95.85
5	97.24

6	96.75
Mean	96.9383
±SD	0.64191
% RSD	0.66

Table 8(a): Observations for change in wavelength

Sr. No.	289 nm		299 nm	
	RT	**Area(mV)**	**RT**	**Area(mV)**
1	3.41	114.90	3.62	109.77
2	3.31	112.85	3.91	110.67
3	3.42	111.75	3.42	110.44
4	3.31	113.87	3.82	108.55
5	3.31	113.88	3.13	106.66
6	3.31	115.77	3.85	107.86
Mean		113.8		108.99
±SD	1.42		1.57	
%RSD	1.25		1.45	

Table 8(b): Observations for change in temperature

Sr. No.	50°C		40°C	
	RT	**Area(mV)**	**RT**	**Area(mV)**
1	3.191	132.84	3.44	122.83
2	3.329	137.32	3.63	123.51
3	2.891	135.27	2.47	125.07
4	3.193	138.24	2.83	121.83
5	3.193	133.34	2.74	123.27
6	2.82	132.38	3.54	126.36
Mean		133.23		123.811
±SD		2.45		1.63
%RSD		1.82		1.32

Table 8(c): Observations for change in flow rate

Sr. No.	0.8 ml/min		1.2 ml/min	
	RT	Area (mV)	RT	Area (mV)
1	3.52	125.72	2.82	126.78
2	3.30	125.35	2.86	124.59
3	3.20	127.49	2.93	126.06
4	3.30	124.35	2.83	123.21
5	3.42	120.44	3.24	119.53
6	3.34	121.21	3.07	123.18
Mean		124.093		123.8
±SD	2.738		2.58	
%RSD	2.21		2.09	

CONCLUSION

The proposed RP-HPLC method was said to rapid, accurate and precise. The % release of drug was found maximum with optimized dissolution parameters and % estimation was nearly 100%. The study of validation parameters must meet the ICH requirements. Hence, this method can be used for the routine analysis of Saroglitazar in bulk and its tablet dosage forms, the results 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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