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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF SAROGLITAZAR IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, rapid and reproducible stability indicating RP - HPLC method was developed and validated for the estimation of Saroglitazar in pharmaceutical dosage forms. Chromatography was performed on a ODS C_{18} (ODS) column (250 mm X 4.6 mm i.d. Particle size 5μ) Acetonitrile: Triethylamine buffer pH4.6:Methanol (70:20:10 v/v) as a mobile phase at a flow rate of 1 ml/ min. The detection was carried out at 292 nm using analytical Tech. PDA 996 Detector. The obtained calibration curve was linear in the concentration range of 10-50μg/ml. The limit of detection and quantification was found to be 1.81 μg/ml and 5.5 μg/ml respectively. It was found that the amount of Saroglitazar present in the Tablet formulation was 100%. The method was validated statistically using SD, %RSD and SE and the values are found to be within the limits. The recovery studies were performed and the percentage recovery was found to be 100 %.

KEYWORDS: Saroglitazar**,** RP-HPLC, PDA detection, Isocratic Elution;Tablet dosage forms.

INTRODUCTION

Saroglitazar is Used mainly for treatment of type -2 diabetes mellitus and dyslipidemia. Saroglitazar IAPUC name is (2*S*)-2-Ethoxy-3-[4-(2-{2-methyl-5-[4-(methylsulfanyl)phenyl]-1*H*-pyrrol-1-yl}ethoxy)phenyl] propanoic acid and molecular formula is $C_{25}H_{29}NO_4S$.[1-7]

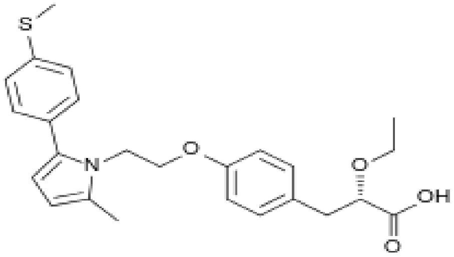

Fig.1 Chemical Structure of Saroglitazar

Literature survey revealed that numerous methods have been reported for estimation of Saroglitazar in pharmaceutical formulations. Present study involves the development of HPLC method[8-12] using simple mobile phase which is sensitive and rapid for quantification of Saroglitazar in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines. The important features and novelty of the proposed method included sonication of sample at ambient temperature treatment with sonication of small amount of powder sample at ambient temperature.

EXPERIMENTAL

Chemicals and reagents

HPLC grade Acetonitrile and water was purchased from SD fine Chemicals (Mumbai, India). Saroglitazar standard sample was provided by sura Labs Pvt (Hyderabad, India). Saroglitazar commercial Tablet formulation (Cadia healthcare limited-Gujarat) was procured from local market. The tablet dosage forms obtained was containing 4 mg of Saroglitazar for oral administration.

Instrumentation and analytical conditions

The HPLC system (WATERS 2695) consisted of a pump.The Analytical column,a ODS C_{18}(250mm×4.6mmi.d.,5µ particle size) was operated at 30ºC temperature (20 ± 1°c). Isocratic elution with Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10) % v/v) was used at a flow rate of 1ml/ min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use (Soltec, Soluzioni tecnologiche, Luglio, Italy). The UV spectrum of Saroglitazar for selecting the working wavelength detection was taken using UV -Visible spectrophotometer (Lab india).

Stock and working standard solutions

Accurately weighed and transferred 100 mg of Saroglitazar working standard into a 100ml of clean dry volumetric flasks, added about 70ml of Diluents and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)
Further pipetted 0.3 ml of the above Saroglitazar stock solutions into a 10ml volumetric flask and diluted up to the mark with diluents.

The solution was further diluted with mobile phase in 10ml volumetric flask to obtain five working standards in the concentration of 10-50 µg/ ml of Saroglitazar. All the solutions were prepared. Before being subjected to analysis, all the working standard solutions were filtered through 0.45 µ filter.

Before injecting solutions, the column was equilibrated for at least 60 min with the mobile phase flowing through the system. The calibration curve was plotted with the five concentrations of the 10-50 µg/ ml working standard solutions. Chromatogram was recorded for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of sample preparation

The contents of twenty commercial tablets (labeled concentration 4 mg of Saroglitazar were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 100 mg of Saroglitazar was quantitatively transfer into a 100ml clean dry volumetric flask and added about 70mL of diluent. The solution was sonicated for 10 min, made volume up to the mark with same solvent mixed well and filtered through 0.45 µ filter. Further pipetted 0.3ml of Saroglitazar above sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. The theoretical Saroglitazar concentration after dilution was 30µg/ ml (100% of Saroglitazar). An aliquot of this solution was a filtered through 0.45 µ filterprior to the injection into the HPLC system. Peak area of Saroglitazar was measured for the determinations.

Validation procedure

The objective of 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 (repeatability and intermediate precision) accuracy, specificity, short term stability and system suitability.

Standard plots were constructed with five concentrations in the range of 10 - 50 µg/ ml of Saroglitazar prepared in 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 repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared Saroglitazar test solution in the same equipment at a concentration of 100% of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of Saroglitazar was determined and precision was reported as % R.S.D. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analyzing samples 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.

Sample solution short term stability was tested at ambient temperature (20 ± 1°C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Screening and optimization

Selection of the detection wavelength

The UV spectra of Saroglitazar in Acetonitrile: Triethylamine buffer pH4.6:Methanol (70:20:10 v/v) mixtures of Water and Acetonitrile in the region between 200 and 400 nm. It shows that at 292 nm, Saroglitazar have maximum absorbance. Hence λ max of Saroglitazar in mobile phase was selected as an optimum detection wavelength for the quantification of Saroglitazar.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. The drug Saroglitazar is a non polar. Non polar compounds preferably analyzed by reverse phase columns. Among C_8 and C_{18}, C_{18} column was selected. Non polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of Acetonitrile: Triethylamine buffer pH4.6:Methanol was selected as mobile phase and the effect of composition of mobile phase on the retention time of Saroglitazar was thoroughly investigated. Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10) were optimized to give symmetric peaks with short run time (Fig 2). A short run time and the stability of peak asymmetry were observed in the ratio of Acetonitrile: Triethylamine buffer pH4.6:Methanol. It was found to be optimum mobile phase concentration.

Chromatographic Conditions

Optimized Method Parameters

Optimized Chromatographic conditions

Mobile phase ratio : Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10)

Column : ODS C_{18} (4.6×250mm) 5µ

Column temperature : 30ºC

Wavelength : 292nm

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 5minutes

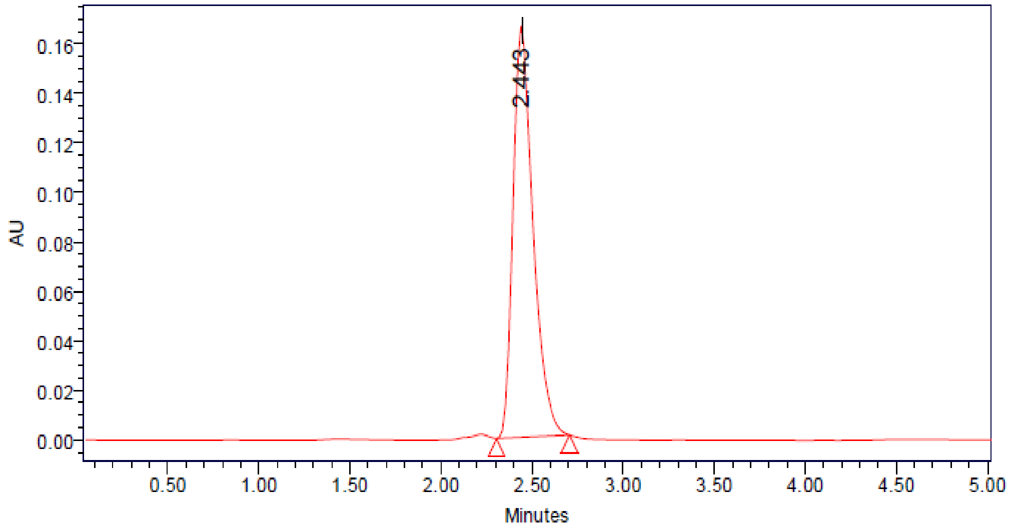

Fig.2 Saroglitazar Optimization of chromatogram

Validation of methods

Linearity

Five point's calibration graphs were constructed covering a concentration range 10-50μg/ ml (Three independent determinations were performed at each concentration. Linear relationships between the of peak area signal of Saroglitazar & the corresponding drug concentration was observed as shown in Fig . The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.9999.The statistical analysis of calibration is shown in table 1.

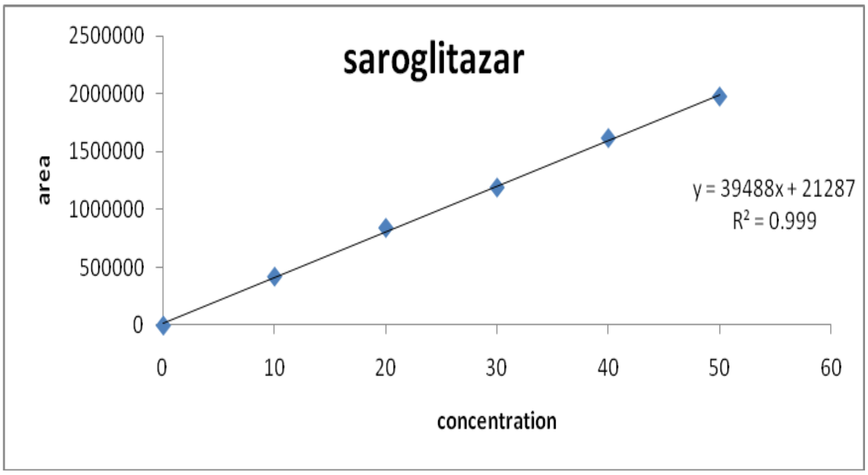

Fig 3 Calibration Graph of Saroglitazar

Precision

The validated method was applied for the assay of commercial tablets containing 4 mg of Saroglitazar Sample was analyzed for six times after extracting the drug similar procedure and assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, was found to be 100 ± 1.02 for showing that the content of Saroglitazar in tablet formulations confirmed to the content requirements (95 - 105 %) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement.

Thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day and on three consecutive days (n=2) indicated a R.S.D. of 0.0455 and 0.03995% respectively. Both values were far below to 2%, the limit percentage indicated a good method precision. The results of analysis are shown in table 2 and table 3.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of Saroglitazar in the real samples. These results are summarized in table 1. The mean recovery data of Saroglitazar in real sample were within the range of 98-102%. Mean % R.S.D. 100%, satisfying the acceptance criteria for the study. It is proves that there is no interference due to excipients used in tablet formulation .Hence the accuracy of the method was conformed.

Table 1 Accuracy study for Saroglitazar (n =9)

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	613351	15	15	100%	100%
100%	1228860	30	30	100%	
150%	1793477	45	45	100%	

* Mean of three observations

Stability: The stability of saroglitazar in standard and sample solutions are determined by storing the solutions at ambient temperature (20 ± 1°C). The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it has noticed the solutions were stable for 48 hrs, as during this time the results did not decrease below 94%. This denotes that Saroglitazar is stable in standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetric factor, tailing factor, HETP and No. of theoretical plates also calculated. It was observed that all the values are within the limits (table 2).

Table 2: Summary for RP-HPLC Method

Parameter	Acceptancecriteria	Results obtained For saroglitazar
System suitability	Theoretical Plates-NLT 2000	5342
	Tailing factor-NMT 2	1.39
	Retention time- NLT 2	2.4
Precision	%RSD- NLT 2	0.11
Linearity	Correlation Coefficient NLT 0.999	0.999
Accuracy	Percentage Recovery 98-102%	100
Limit of detection		1.81µg/ml
Limit of quantitation		5.50 µg/ml

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation parameters for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Saroglitazar in tablet formulation.

Forced Degradation Studies

Sample Preparations To Perform Forced Degradation Studies

Acid degradation: Accurately weighed 10mg of equivalent weight of Saroglitazar sample into a 10ml clean dry volumetric flask and added about 3mL of 0.1N Hcl and kept side for 3hours and then 3mL of 0.1N NaOH solution is added to neutralize the solution and then made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3 ml of above stock solution into a 10ml volumetric flask (it contains Saroglitazar) and diluted up to the mark with diluent.

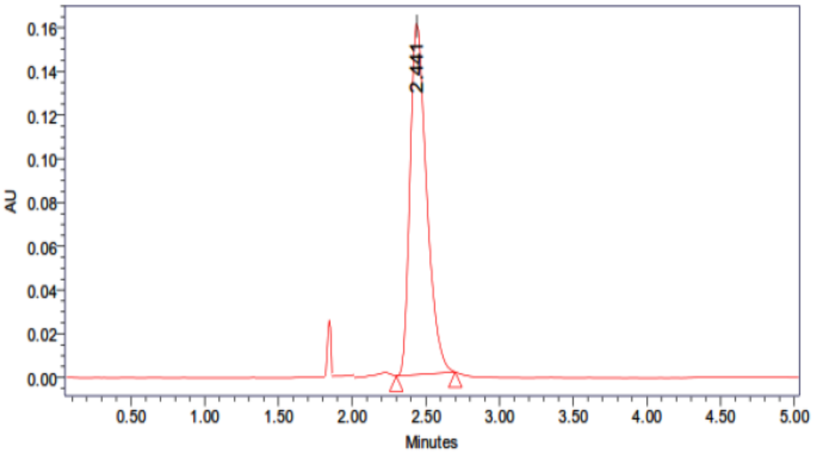

Figure 4: showing acid degradation for saroglitazar

Alkaline degradation

Accurately weighed 10 mg equivalent weight of Saroglitazar sample into a 10mL clean dry volumetric flask and added about 3mL of 0.1N NaOH and kept side for 3hours and then added 3mL of 0.1N Hcl solution to neutralize the solution and made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Saroglitazar) and diluted up to the mark with diluent.

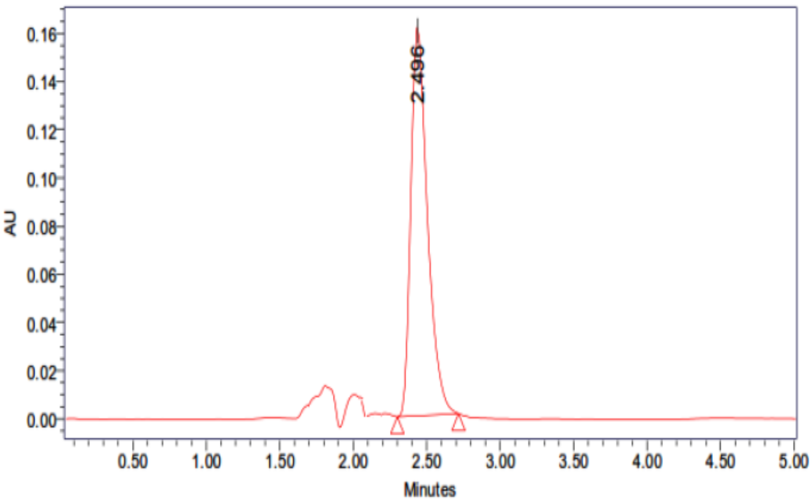

Figure 5 showing Alkaline degradation for saroglitazar

Peroxide degradation

Accurately weighed 10 mg equivalent weight of Saroglitazar sample into a 10mL clean dry volumetric flask and added about 3mL of Hydrogen peroxide solution and kept side for 3hours and made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Saroglitazar) and diluted up to the mark with diluent.Fig 6.

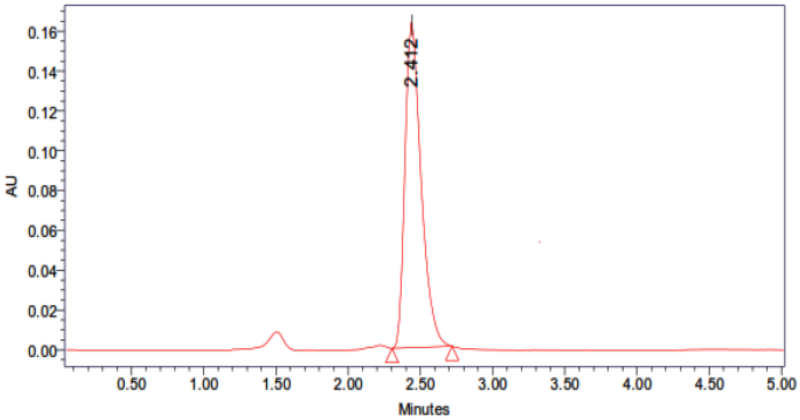

Figure 6 showing peroxide degradation for saroglitazar

Thermal degradation

Accurately weighed 10 mg equivalent weight of Saroglitazar sample into a 10mL clean dry volumetric flask and exposed to heat at 80-90°c for 3hours and then made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Saroglitazar) and diluted up to the mark with diluent.Fig 7.

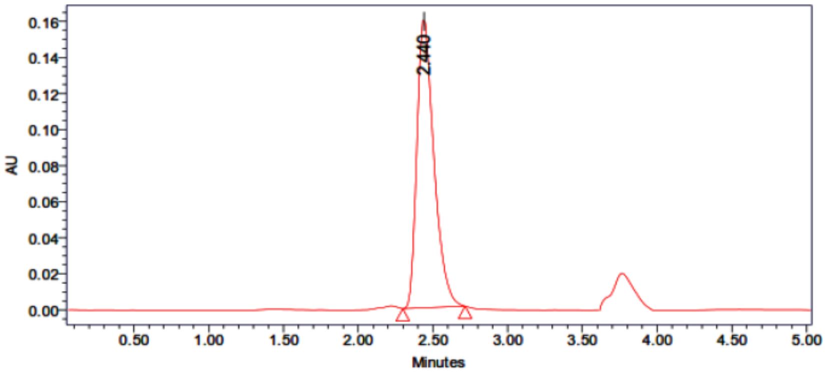

Figure 7 showing Thermal degradation for saroglitazar

Photolytic degradation

Accurately weighed 10 mg equivalent weight of Saroglitazar sample into a 10mL clean dry volumetric flask and exposed to sunlight for 3hours and made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Saroglitazar) and diluted up to the mark with diluent. Figure: 8

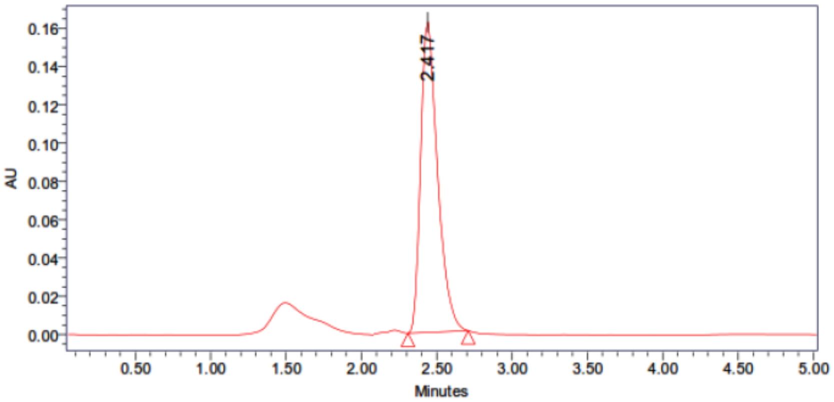

Figure 8: showing Photolytic degradation for saroglitazar

Table 3: Results for degradation studies

S. No	Type of degradation	Concentration of sample (µg/ml)	Area of sample	Assay content (% w/w)
1	Acid (0.1N HCl)	30 µg/ml	1206721	100%
2	Base (0.1N NaOH)	30 µg/ml	1214352	100%
3	Peroxide (3% H_20_2)	30 µg/ml	1145269	94%
4	Thermal (at 60^0 c)	30 µg/ml	1195615	99%
5	Photolytic (sunlight)	30 µg/ml	1185917	98%

SUMMARY AND CONCLUSION

SUMMARY

RP-HPLC method was developed for estimation of Saroglitazar in bulk and Pharmaceutical dosage form. Chromatographic separation was, carried out on a ODS C18 (4.6×250mm) 5µ column with mobile phase comprising mixture of Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10) at a flow rate of 1.0ml/min, the detection was carried out at 292nm

CONCLUSION

The proposed RP-HPLC method was found to be precise, specific, accurate, rapid and economical for estimation of Saroglitazar in bulk and in its Pharmaceutical dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and the % RSD values were with in 2 and the method was found to be precise. This method can be used for routine determination of Saroglitazar in bulk and in Pharmaceutical dosage forms.

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