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Research Article

# STABILITY INDICATING CHIRAL HPLC METHOD FOR THE ESTIMATION OF PIOGLITAZONE ENANTIOMERS IN PHARMACEUTICAL FORMULATION

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**ABSTRACT**

**Objective:** A stability indicating chiral high performance liquid chromatographic (HPLC) method was developed and validated for the separated (S) and (R) pioglitazone in raw material and its determination in the presence of degradation products formed during forced degradation studies.

**Methods:** In the present study, an isocratic normal phase-HPLC method was developed with stationary phase as ACI Cellu 1 (150 mm × 4.6 mm i.d., 5 µ) column and n-hexane: N-propyl alcohol (80:20, V/V) as mobile phase. The entire study was performed using 1.0 ml/minute as flow rate and the detection wavelength at 233 nm. The pioglitazone (R and S) was exposed to various stress condition such as hydrolytic (acid and base), neutral, oxidative, and photolytic. The stressed samples were analyzed by the proposed method.

**Result:** The described method was linear over the range of 5-15 µg/ml for R-pioglitazone and 4-14 µg/ml for S-pioglitazone. The limit of detection and limit of quantification of S-pioglitazone and R-pioglitazone were found to be 1.4 µg/ml and 4.26 µg/ml, respectively. The recovery study of S and R-Pioglitazone from tablets formulation ranged from 97.14% to 100.04%, respectively.

**Conclusion:** The developed method can be applied in the quality control of drug products.

**Keywords:** Stability-indicating method, Validation, Chiral, Pioglitazone.

## INTRODUCTION

Pioglitazone is chemically known as (RS)-5-(4-[2-(5-ethylpyridin 2yl)ethoxy]benzyl)thiazolidine-2,4-dione. Pioglitazone is the thiazolidinedione-ring with the chiral center [1]. Glitazones are potent insulin sensitizers. In recent years, direct resolution of enantiomers without prior derivatization has come into use as alternative procedures to indirect methods [2-6]. These methods involve the use of chiral stationary phases that form transient diastereomeric complexes with solute enantiomers. Investigation of the single enantiomers in a racemic mixture may be valuable in order to investigate whether the single enantiomers demonstrate the difference in pharmacological effect and/or effect ratio than the other. However, in order to perform the necessary investigations for making decisions regarding this, it is required to test the enantiomers separately. So, the pure enantiomers should either be synthesized (if possible) or purified from the racemic mixture using preparative separation methods. The aim of the current study is to demonstrate that it is possible to separate enantiomers from a racemic mixture of pioglitazone with respect to further investigations on these important potent insulin sensitizers, which in turn leads to a possible better treatment of diabetes. Development of a better and safer drug product may be considered if one of the enantiomers has a significantly better effect than the other. Literature survey revealed that only high performance liquid chromatographic (HPLC) methods for its determination in pharmaceuticals and in human serum have been reported [7-10].

The stability-indicating assay methods (SIAMS) are employed for the analysis of stability samples in the pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishing SIAM has become mandated [11]. The guidelines explicitly require the conduct of forced decomposition studies under a variety of conditions, such as pH, light, oxidation, dry heat, etc. and separation of the drug from degradation products. The developed method is expected to allow analysis of individual degradation products. The ICH guideline Q1A (R2) [12] emphasizes that the testing of those features, which are susceptible to change during storage and are likely to influence quality, safety and efficacy of the drug, must be examined by validated stability indicating testing method. As per Q1 (R2) information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways, the intrinsic stability of the molecule, and validate the stability indicating power of the developed analytical procedures. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.

From the literature review, it is observed that no stability indicating assay method for the determination of pioglitazone enantiomers was available keeping in the view of the susceptibility of pioglitazone enantiomers under a variety of conditions. It was felt that an (SIAMS) that separates the drug enantiomers from their degradation products formed need to be developed.

## METHODS

### Solvents and chemicals

n-Hexane (HPLC grade), 1-propyl alcohol were supplied by Qualigens fine chemicals and S.D. fine chemicals. Reference standards of Pioglitazone enantiomers were procured from Sigma-Aldrich limited, Mumbai, India. Working standard of pioglitazone RS (99.37%) was obtained as gift sample from Micro Labs Limited, Hosur, Tamil Nadu, India. Commercially available tablets Pioglitazone were purchased commercially from the local market, Ooty, Tamil Nadu, India.

### Apparatus and instrument conditions

Chromatographic separation and quantitative determination were performed using a HPLC containing a Shimadzu gradient pump (LC-20 AD solvent delivery system), an ultraviolet detector (Shimadzu). The

stationary phase, ACI Cellu 1 (150 mm × 4.6 mm i.d., 5 µ particle size) was used. The mobile phase consisted of 80 volumes of n-hexane and 20 volumes of 1-propyl alcohol. The flow rate of the mobile phase was 1.0 ml/minute, with the detection wavelength at 233 nm.

**Preparation of standard solution**

The stock solutions containing 1 mg/ml of R and S form of pioglitazone were prepared in methanol. These stock solutions were stored in light-resistant containers. Aliquots of R-pioglitazone (5-15 µg/ml) and S-pioglitazone (4-14 µg/ml) were prepared in the mobile phase for analysis.

**Preparation of sample solution**

Twenty tablets were weighed, to determine the average weight and finely powdered. The powder equivalent to 5 mg of R and S form of pioglitazone was accurately weighed and transferred into a 10 ml volumetric flask. To this 5 ml of mobile phase was added and sonicated for 10 minutes. The resulting solution was made up to 10 ml with mobile phase and filtered using Whatman filter paper No. 42. The components R and S enantiomers of pioglitazone from one formulation (Pepar tablet containing 15 mg of pioglitazone) were extracted in the mobile phase. The standard and sample solutions were analyzed by the optimized chromatographic conditions, the chromatograms were recorded.

**Forced degradation study of pioglitazone**

In order to establish whether the analytical method and the assay were indicating stability, pure active pharmaceutical ingredient (API) of both pioglitazone enantiomers was forced under various stress conditions to conduct degradation studies. As the two enantiomers are freely soluble and stable in methanol, it was used as a co-solvent in all the forced degradation studies. All solution used in forced degradation studies, were prepared by dissolving API in small volume of methanol and later diluted with aqueous hydrogen peroxide, distilled water, aqueous hydrochloric acid or aqueous sodium hydroxide to achieve a concentration of 100 mcg/ml of pioglitazone. Photodegradation studies were performed in methanol. The solutions were exposed to sunlight during the day time for 4 days. The resultant solutions were analyzed every day, control samples which were protected from light with aluminum foil were also placed in the daylight concurrently, and studied for any degradation with the optimized chromatographic condition.

## RESULTS AND DISCUSSION

**Optimized of chromatographic conditions**

A solvent combination of n-hexane: N-propyl alcohol (80:20, % V/V) on a ACI Cellu 1 (150 mm × 4.6 mm i.d., 5 µ) as stationary phase gave satisfactory separation of R and S pioglitazone and their degradation products formed under various stress conditions. The detection was carried out at 233 nm with a flow rate of 1.0 ml/minute. The retention times of pioglitazone R and S were observed to be 3.6 minutes and 4.6 minutes, respectively (Figs. 1 and 2).

The HPLC studies of samples obtained on stress testing of pioglitazone enantiomers under different conditions suggested the following degradation behaviors (Table 1). Complete degradation of R and S pioglitazone was found with either 1N HCl or 1N NaOH hence 0.1N HCl or 0.1N NaOH was used for the degradation studies after 1, 2, 4, 8, 12 and 24 hrs (Figs. 3-5). Pioglitazone enantiomers were found to be stable under acidic condition up to 6 hrs. Only around 4% of the pioglitazone enantiomers were degraded through 24 hrs, and the main analyte were eluted at 3.6 and 4.6 minutes during HPLC analysis. In alkaline stress conditions, only 25% decomposition of the pioglitazone enantiomers was degraded as compared to the standard solution of the drug. The drug enantiomers were completely degraded when utilized 30% $H_2O_2$ but falling by 8% by using 3% $H_2O_2$ through 24 hrs (Fig. 3). Under neutral conditions R and S pioglitazone enantiomers were found to be stable up to 24 hrs. Moreover very slight degradation was observed on exposure of solution and solid drug powder to sunlight. Temperature stress studies R and S pioglitazone enantiomers were found to be stable at 24 hrs.

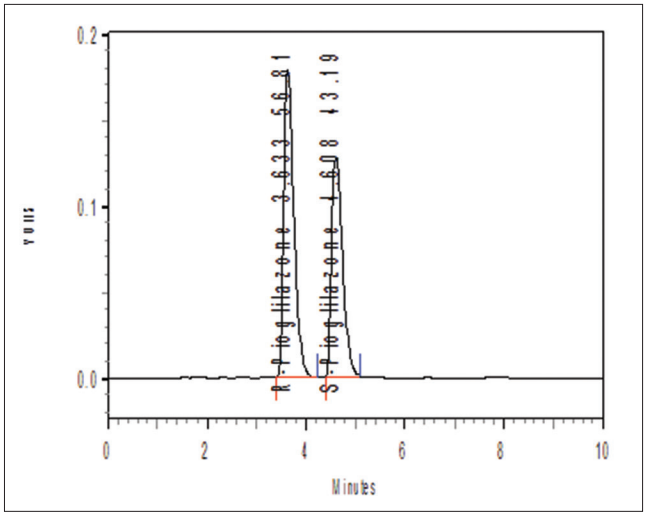


**Fig. 1: Typical high performance liquid chromatographic chromatogram of pioglitazone standard solution**

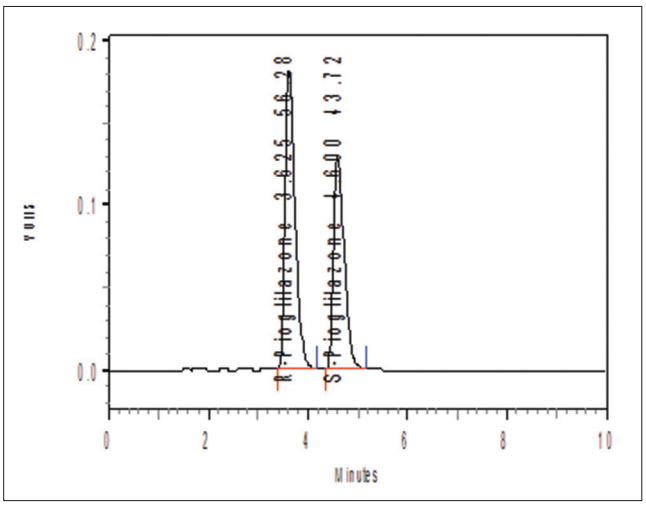


**Fig. 2: Typical high performance liquid chromatographic chromatogram of pioglitazone sample I solution**

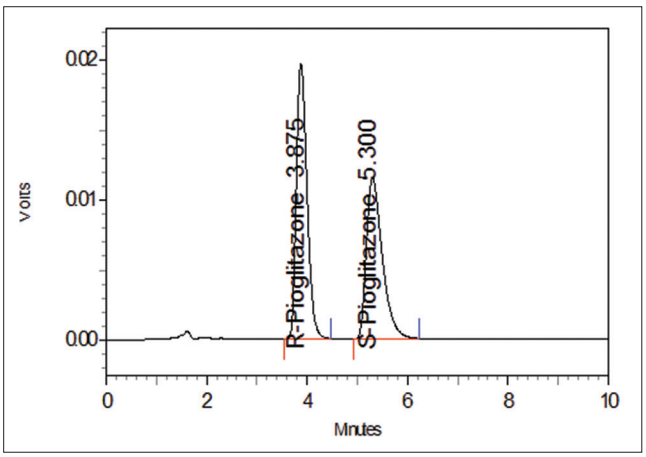


**Fig. 3: Typical high performance liquid chromatographic chromatogram of oxidative degradation sample of pioglitazone (R band S) with 0.1N HCl at 24 hrs**

The calibration curves of R-pioglitazone and S-pioglitazone were linear in the range of 5-15 µg/ml and 4-14 µg/ml respectively. Linear

**Table 1: Stress degradation studies of pioglitazone RS**

| S. No | Time (hrs) | Acid hydrolysis (% degradation) | | Basic hydrolysis (% degradation) | | Neutral degradation (% degradation) | | Oxidative degradation (% degradation) | | Photo degradation (% degradation) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | S | R | S | R | S | R | S | R | S |
| | | 1N HCl | | 1N NaOH | | | | 30% $H_2O_2$ | | Powder form | |
| 1 | 0 | 0 | 0 | 0 | 0 | | | 0 | 0 | | |
| 2 | 2 | 28.65 | 23.16 | 36.56 | 38.86 | | | 25.53 | 23.76 | 24 hrs | |
| | | 0.1 N HCl | | 0.1N NaOH | | | | 3% $H_2O_2$ | | 0.21 | 0.24 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 2 | 2 | 0.43 | 1.93 | 19.67 | 20.44 | 0.17 | 0.27 | 0.02 | 0.99 | | |
| 3 | 4 | 0.53 | 1.98 | 20.26 | 20.62 | 0.19 | 0.47 | 0.39 | 1.02 | Solution form | |
| 4 | 6 | 1.78 | 1.96 | 20.27 | 23.63 | 0.57 | 0.50 | 1.75 | 3.70 | 24 hrs | |
| 5 | 8 | 2.44 | 2.13 | 21.41 | 24.26 | 0.64 | 0.52 | 2.02 | 5.78 | 0.16 | 0.14 |
| 6 | 12 | 2.76 | 2.61 | 25.02 | 25.02 | 0.66 | 0.57 | 7.25 | 7.18 | | |
| 7 | 24 | 3.87 | 3.22 | 25.20 | 25.82 | 0.75 | 0.62 | 7.86 | 7.51 | | |

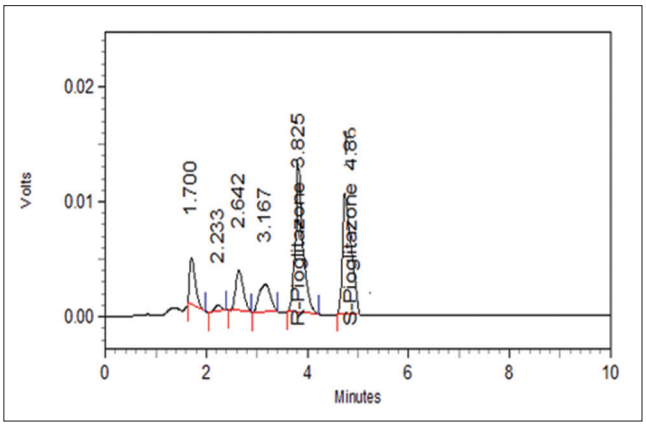


**Fig. 4: Typical high performance liquid chromatographic chromatogram of oxidative degradation sample of pioglitazone (R and S) with 0.1N NaOH at 24 hrs**

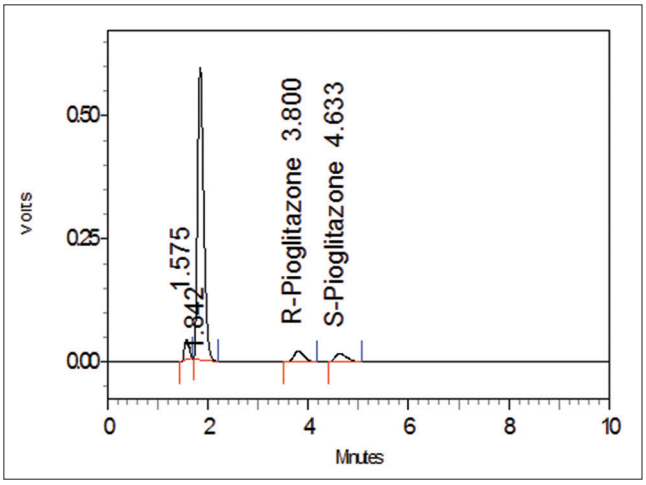


**Fig. 5: Typical high performance liquid chromatographic chromatogram of oxidative degradation sample of pioglitazone (R and S) with 3% $H_2O_2$ at 24 hrs**

regression equation and correlation coefficient are shown in Table 2. The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation and % relative standard deviation (RSD) were calculated. The % RSD values were found to be <2% revealed that, the method was precise. The accuracy of the optimized methods was determined by absolute recovery experiments. An analysis of the results showed that the percentage recovery values were close to 100 % thus establishing that the developed method is

**Table 2: Linearity and range for S and R pioglitazone enantiomers by HPLC**

| S. No | R-piog alitazone | | S-pioglitazone | |
|---|---|---|---|---|
| | Concentration µg/ml | Peak area | Concentration µg/ml | Peak area |
| 1 | 5 | 326557 | 4 | 263294 |
| 2 | 7.5 | 489845 | 6.5 | 427865 |
| 3 | 10 | 653119 | 9 | 592431 |
| 4 | 12.5 | 816398 | 11.5 | 756990 |
| 5 | 15 | 979679 | 14 | 921537 |

HPLC: High performance liquid chromatographic

accurate and reliable (Table 3). Detection limits and quantification limits of R-pioglitazone and S-pioglitazone were found to be 1.4 µg/ml and 4.26 µg/ml respectively (Table 1). No marked changes in the chromatogram occurred on changing the instrument; operator and chromatographic conditions indicated that the developed method was rugged and robust. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. Signal to noise ratio of 3 and 10 are generally considered as the limit of detection (LOD) and limit of quantification (LOQ), respectively. The LOD and LOQ of R and S pioglitazone obtained by the method were 1.4 µg/ml and 4.26 µg/ml (Table 4). The values obtained demonstrated the suitability of the system for the analysis of pioglitazone enantiomers in a pharmaceutical formulation. Additionally, the developed HPLC method was also applied for the estimation of pioglitazone in pharmaceutical formulations.

**Method validation**

The normal phase HPLC method was validated according to ICH guidelines. The linearity of R and S pioglitazone were studied by preparing and assaying a series calibration standard at six different concentrations within the range from 5 to 15 µg/ml of R-pioglitazone and 4-14 µg/ml of S-pioglitazone. A good linear relationship was observed between the peak area ratios of R and S pioglitazone and the concentrations in the investigated concentration range.

The specificity of the method was ascertained by analyzing the standards and the samples. The peaks of R and S pioglitazone in samples were confirmed by comparing the retention time and spectra of the standards. Six injections at three different concentrations of R-pioglitazone (5, 10, 15 µg/ml) and S-pioglitazone (4, 9, 14 µg/ml) enantiomers were made and analyzed to examine the precision of the method. The mean peak area, standard deviation and % RSD were calculated.

The accuracy of the method was determined by recovery of pioglitazone from its pharmaceutical formulation. The recovery test was performed at three levels, namely, 80%, 100%, and 120% of the nominal

**Table 3: Results of analysis of drug products and recovery studies for S and R pioglitazone enantiomers by HPLC**

| Sample | Label claim (mg) | Amount present (mg/tablet)±% RSD* | | | % Label claim | | | % Recovery±%RSD* |
|---|---|---|---|---|---|---|---|---|
| | | R and S | R | S | R and S | R | S | |
| I | 15 | 15.11±0.25 | 8.34±0.39 | 6.75±0.45 | 100.70±0.93 | 55.66±0.67 | 45.03±0.64 | 100.04±0.92 |
| II | 15 | 15.22±0.52 | 8.47±0.76 | 6.74±0.65 | 101.48±0.47 | 56.50±0.98 | 44.97±0.18 | 98.40±0.51 |
| III | 30 | 30.45±0.44 | 16.88±0.89 | 13.56±0.84 | 101.51±0.34 | 56.29±0.63 | 45.21±0.32 | 97.14±0.96 |
| IV | 30 | 30.39±0.97 | 16.87±0.51 | 13.52±0.34 | 101.31±0.68 | 56.23±0.28 | 45.08±0.51 | 99.88±0.53 |

HPLC: High performance liquid chromatographic, RSD: Relative standard deviation

**Table 4: System suitability studies for estimation of S and R pioglitazone enantiomers by HPLC**

| S. No | Parameters | R - pioglitazone | S - pioglitazone |
|---|---|---|---|
| 1 | Linearity range | 5-15 µg/ml | 4-14 µg/ml |
| 2 | Regression equation Y=mx+c | y=58204x+25747 | y=47469x+65284 |
| 3 | Correlation coefficient | 0.996 | 0.996 |
| 4 | Theoretical plate/meter | 54082 | 54365 |
| 5 | Resolution factor | 1.85 | |
| 6 | Asymmetric factor | 1.02 | 1.01 |
| 7 | LOD (µg/ml) | 1.4 | 1.4 |
| 8 | LOQ (µg/ml) | 4.26 | 4.26 |

HPLC: High performance liquid chromatographic, LOD: Limit of detection, LOQ: Limit of quantification

concentration of pioglitazone, during degradation studies. Triplicate samples were prepared for each recovery level. The solutions were analyzed, and the percentage of recoveries was calculated from the calibration curves.

The ruggedness of the proposed method was determined by carrying out the experiment on different operators. Robustness of the method was determined by making small changes in the chromatographic conditions such as flow rate, and mobile phase composition were deliberately varied and resolution between the two peaks is found >2.0 and % RSD of all the compounds were within the limit and this illustrates the robustness of the method as stated in ICH guidelines.

## CONCLUSION

A highly specific stability-indicating chiral HPLC assay method was developed for the quantification of pioglitazone enantiomers in the presence of their degradation products. The enantio- the separation was carried out by the use of the cellulose-based chiral column. The total run time for the developed method is 10 minutes. The method provides good sensitivity and excellent precision and reproducibility.

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