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Research Article

A NEW VALIDATED STABILITY-INDICATING DIRECT HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF ROSIGLITAZONE ENANTIOMERS IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

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ABSTRACT

Objective: In the present study, an isocratic chiral reverse-phase high-performance liquid chromatography method was developed and the resolution of the drug and complete separation from its degradation products were successfully achieved.

Methods: An isocratic method developed with a Phenomenex Lux 5 µ Cellulose 1 (150 mm×4.6 mm i.d., 5 µ) using UV detector at wavelength of 220 nm, with a mobile phase consisting of methanol:0.1% diethylamine (60:40% v/v) and a flow rate of 1 ml/min. The drug was subjected to alkaline, acidic, neutral, oxidative, and photolytic to apply stress conditions. The stressed samples were analyzed by the proposed method.

Results: The described method was linear over the range of 3–7 µg/ml for R-enantiomer and 9–21 µg/ml of S-enantiomer, respectively. The limit of detection and limit of quantification of R and S enantiomers were found to be 0.56 µg/ml and 0.18 µg/ml, respectively.

Conclusion: The method provides good sensitivity and excellent precision and reproducibility. The developed method can be applied in the quality control of drug products.

Keywords: Stability-indicating method, Validation, Chiral, Rosiglitazone.

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INTRODUCTION

The stability-indicating assay is a method applied to ensure the stability of a drug. The presence of impurities and degradation products can change chemical, pharmacological, and toxicological properties of drugs having significant impact on their quality and safety. Since drugs are, especially, sensitive to environmental factors, strict storage conditions are necessary [1]. The ICH guideline Q1A on stability testing of new drug substances suggests that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety, and efficacy must be done by validated stability-indicating methods. Stress testing should be carried out on a drug to establish its inherent stability characteristics and to support the suitability of the proposed analytical method. It is also suggested that stress testing should include the effects of temperature, susceptibility across a wide range of pH values, as well as oxidative and photolytic conditions [2].

Like stability-indicating study, the enantioseparation has a great impact on the pharmacological and toxicological properties of chiral drugs in terms of the control of individual variability in clinical responses between the enantiomers. A prerequisite for progress in acquiring knowledge of stereo-specific pharmacodynamic and pharmacokinetics of optical isomers is the development of an enantioselective analytical methodology. High-performance liquid chromatography (HPLC) is one of the famous analytical techniques commonly employed in conducting stability studies and in chiral separation [3-4]. HPLC has gained popularity due to its high-resolution capacity, speed, sensitivity, and specificity. Separation of enantiomers by HPLC can be achieved using either chiral stationary phases (CSPs) or chiral additives to the mobile phase. The most common HPLC approach for resolving enantiomers involves the use of CSPs [4-9].

Rosiglitazone hydrochloride is chemically known as rosiglitazone (RS)-5-[4-(2-[methyl (pyridin-2-yl)amino]ethoxy)benzyl]thiazolidine-2,4-dione (Fig. 1) has an oral hypoglycemic agent used in Type II diabetes. It targets insulin resistance. It is highly selective and potent against for the peroxisome proliferators-activated receptor (PPAR)-gamma. The activation of PPAR-γ nuclear receptor receptors is said to regulate the transcription of insulin-responsive genes, involved in the control of glucose production, transport, and utilization [10,11].

From the literature review, it is observed that no stability-indicating assay methods (SIAMs) for the determination of rosiglitazone enantiomers was available keeping in the view of susceptibility of rosiglitazone enantiomers under variety of conditions. It was felt that SIAMs that separate the drug enantiomers from their degradation products formed need to be developed. Hence, the aim of this study is to establish a validated stability-indicating chiral HPLC method for rosiglitazone enantiomers using Phenomenex Lux Cellulose 1 column. None of the reported analytical methods described stability-indicating method for the reverse-phase (RP) chiral HPLC method of rosiglitazone enantiomers in the presence of its degradation products. To the best of our knowledge, this is the first report of stability-indicating method for the chiral determination of rosiglitazone enantiomers.

RESEARCH METHODS

Tools and materials

Materials

Solvents and chemicals

Rosiglitazone RS (98.67%) was obtained as gift sample from Sun Pharmaceutical Industries Ltd., Bharuch, Gujarat, India. Methanol,

acetonitrile, and isopropyl alcohol (HPLC grade) were supplied by Merck, diethylamine and trifluoroacetic acid from S.D. Fine Chemicals. Commercially available tablets rosiglitazone were purchased commercially from the local market, Udhagamandalam, Tamil Nadu, India.

Instrumentation

Chromatographic separation and quantitative determination were performed using an HPLC system, from Shimadzu (Kyoto, Japan) equipped with LC-10 AT-VP solvent data station delivery system, an SPD M10 A UV detector, LC-2010 an HT autosampler with loop volume of 100 µL, and the class VP data station was used. The stationary phase Phenomenex Lux Cellulose 1 (×250 4.6mm i.d, 5 µ) column was used for the enantiomeric separation of rosiglitazone and the mobile phase consists methanol:0.1% diethylamine (60:40% v/v) and a flow rate of 1 ml/min with detection wavelength at 220 nm.

Selection of wavelength

About 10 µg/ml solution of rosiglitazone in methanol was prepared and recorded UV spectrum by scanning the solution in the range of 200 nm–400 nm. Wavelength of 220 nm was selected from UV spectrum (Fig. 2) at which the maximum absorbance showed for rosiglitazone hydrochloride.

Preparation of stock and standard solution

Each 10 mg of R and S form of rosiglitazone was taken into 10 ml volumetric flasks. To this, 5 ml of methanol was added and sonicated for 10 min until a clear solution was obtained. The resulting solution was made up to 10 ml with methanol (standard solution A). These stock solutions were stored in light-resistant containers. Aliquots of mixed standards of rosiglitazone R and S (10 µg/ml**)** were prepared in mobile phase for analysis. The standard solution was prepared and injected and the chromatogram was recorded (Fig. 3).

Forced degradation studies

Pure active pharmaceutical ingredient of rosiglitazone hydrochloride was forced under various stress conditions to conduct degradation studies. The forced degradation of the drugs can be achieved by the following stress conditions:

- Hydrolysis under acidic and alkaline conditions
- Hydrolysis under neutral conditions
- Oxidative degradation
- Photolytic degradation.

Rosiglitazone standard is freely soluble and stable in methanol, so methanol was used as a cosolvent in all the forced degradation studies. The solutions were prepared by dissolving the active pharmaceutical ingredient in small volume of methanol and later diluted with aqueous sodium hydroxide, aqueous hydrochloric acid, distilled water, and aqueous hydrogen peroxide to achieve a concentration of 10 µg/ml of rosiglitazone photodegradation study also performed using methanol.

Base degradation

About 1 ml of standard stock solution was taken into 10 ml volumetric flask and volume was made up with 0.1 N sodium hydroxide. The solution was kept at room temperature for 2 h. The samples were analyzed after 2 h. 1 ml of sample was diluted to 10 ml with mobile phase. The samples were injected and the chromatograms were recorded (Fig. 4). Further, to increase the extent of degradation, the standard solution was treated with 1 M sodium hydroxide and the solution was kept at room temperature for 24 h. 1 ml aliquots of the samples were withdrawn at 1, 2, 4, 8, 12, and 24 h. The samples were further diluted and analyzed by the optimized chromatographic conditions. The solutions were injected and the chromatograms were recorded.

Acid degradation

About 1 ml of standard stock solution was taken into 10 ml volumetric flask and volume was made up with 0.1 N hydrochloric acid. The solution

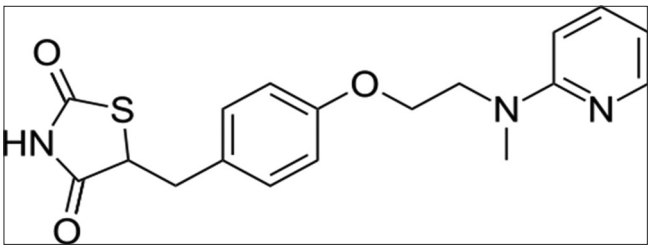

Fig. 1: Chemical structure of rosiglitazone

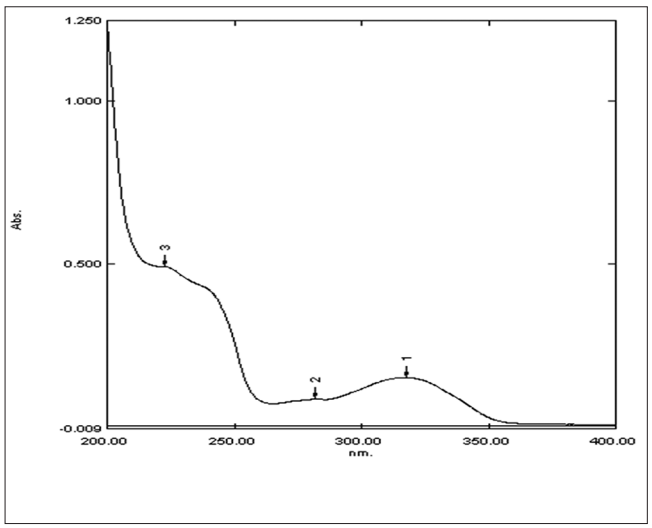

Fig. 2: UV spectrum of rosiglitazone (abs=absorbance)

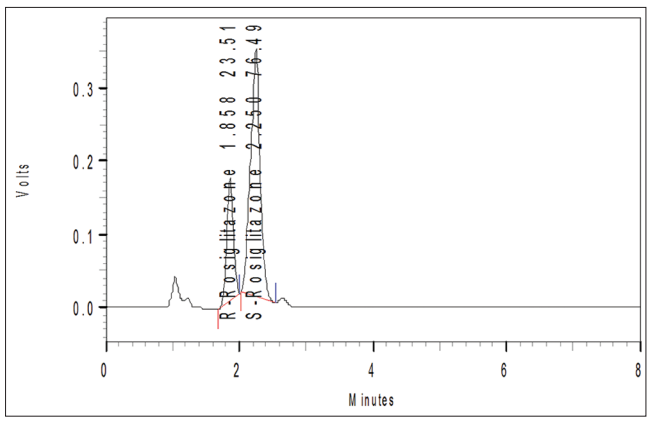

Fig. 3: Typical high-performance liquid chromatography standard chromatogram of rosiglitazone

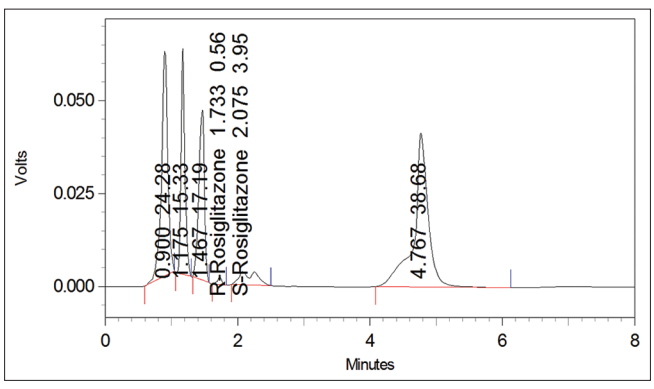

Fig. 4: Typical high-performance liquid chromatography chromatogram of basic degradation sample of R and S rosiglitazone with1 N NaOH at 0 h

was kept at room temperature for 2 h. The samples were analyzed after 2 h. 1 ml of sample was diluted to 10 ml with mobile phase. The samples were injected and the chromatograms were recorded. Further, to increase the extent of degradation, the standard solution was treated with 1 M hydrochloric acid and the solution was kept at room temperature for 24 h. 1 ml aliquots of the samples were withdrawn at 1, 2, 4, 8, 12, and 24 h. The samples were further diluted and analyzed by the optimized chromatographic conditions. The solutions were injected and the chromatograms were recorded.

Neutral degradation

About 1 ml of 1000 µg rosiglitazone sample was taken into 10 ml volumetric flask and diluted with Milli Q water, contents were mixed well. The solution was kept at room temperature for 24 h and 1 ml aliquots of the solutions were withdrawn at 2, 4, 6, 8, 12, and 24 h. 1 ml of this solution was taken into 10 ml volumetric flask and then diluted to 10 ml with mobile phase. The solutions were injected and the chromatograms were recorded (Fig. 5).

Oxidative degradation

About 1 ml of 1000 µg rosiglitazone sample was taken into 10 ml volumetric flask and diluted with 30% hydrogen peroxide, contents were mixed well. The solution was kept at room temperature for 24 h and 1 ml aliquots of the solutions were withdrawn at 2, 4, 6, 8, 12, and 24 h. 1 ml of this solution was taken into 10 ml volumetric flask and then diluted to 10 ml with mobile phase. The solutions were injected and the chromatograms were recorded.

Photolytic degradation

About 1 ml of 1000 µg rosiglitazone sample was taken into 10 ml volumetric flask, and diluted with mobile phase and kept in UV chamber for 24 h, and 1 ml aliquots of the solutions were withdrawn at 2, 4, 6, 8, 12, and 24 h. 1 ml of this solution was taken into 10 ml volumetric flask and then diluted to 10 ml with mobile phase. The solutions were injected and the chromatograms were recorded.

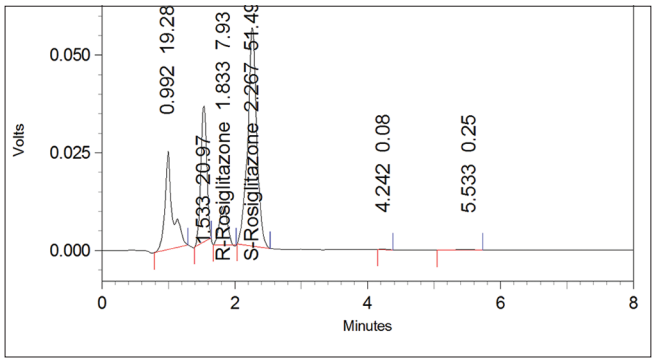

Fig. 5: Typical high-performance liquid chromatography chromatogram of neutral degradation sample of R and S rosiglitazone with neutral at 24 h

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

HPLC method carried out in this study, aimed at developing a chromatographic system, capable of eluting and resolving rosiglitazone enantiomers from their degradation products. The preliminary investigations were directed toward the effect of various factors on the system. The factors assessed include the type of column and the composition of mobile phase. The samples were initially analyzed using a mobile phase consisting of ACN, 20 mM ammonium bicarbonate (pH: 4.6) adjusted with trifluoroacetic acid, and methanol (55:15:30 v/v/v). Under this condition, no separation of rosiglitazone enantiomers was achieved. Partial separation was observed when triethylamine was added to the mobile phase. To improve the peak shape, diethylamine was used instead of triethylamine. Various percentages of methanol and diethylamine were tested to achieve the optimum separation of the two enantiomers.

Finally, the optimized chromatographic conditions are with the mobile phase consisting of methanol:0.1% diethylamine (60:40% v/v) on a Phenomenex Lux Cellulose 1 (250×4.6 mm i.d, 5 µ) column gave a satisfactory separation of rosiglitazone enantiomers and their degradation products formed under various stress conditions. The detection was carried out under 220 nm with flow rate of 1 ml/min. The retention times of R enantiomers and S were observed to be 1–8 min and 2.2 min, respectively.

Forced degradation studies

HPLC studies of samples obtained on stress testing of rosiglitazone enantiomers under different conditions suggested the following degradation behaviors (Table 1). Complete degradation of R-rosiglitazone and S was found with 1 N NaOH and 30% hydrogen peroxide (Fig. 4) around 24.96, 24.97, and 28.79, 34.80% of degradation after 24 h. Rosiglitazone enantiomers were found to be stable under acidic condition; around 9.46% and 9.89% were degraded through 24 h, and the main analyte was eluted at 1.8 and 2.2 min during HPLC analysis. In neutral stress condition, only 4.24% and 5.57% were degraded through 24 h as compared to standard drug (Fig. 5). In photodegradation, 0.79% and 2.05% were degraded through 24 h, respectively. The drug was degraded more in alkaline and oxidation condition.

Method validation

RP-HPLC method was validated according to the ICH guidelines. The calibration curves of enantiomer I and enantiomer II were linear in the range of 56–84 µg/ml and 52–78 µg/ml, respectively. The regression equation and correlation coefficient are shown in Fig. 6. The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation, and percentage relative standard deviation (RSD) were calculated. The percentage RSD values were found to be <2% revealed that the method was precise. The accuracy of the optimized method was determined by absolute recovery experiments. An analysis of the results showed that the percentage recovery values were close to 100%, thus

Table 1: Results of stress degradation studies of rosiglitazone enantiomers by HPLC

S. No.	Time (h)	Basic hydrolysis (% degradation) 1 N NaOH		Acid hydrolysis (% degradation) 1 N HCL		Neutral degradation (% degradation) H_2O		Oxidative degradation (% degradation) 30% H_2O_2		Photodegradation (% degradation)	
		I	II	I	II	I	II	I	II	I	II
1	0	0	0	0	0	0	0	0	0	0	0
2	2	22.83	28.51	2.57	2.33	0.55	0.89	23.40	29.04	0	0
3	4	22.97	28.87	3.46	4.57	0.88	1.35	24.01	30.31	0	0
4	6	23.05	29.17	4.69	5.13	1.33	2.57	24.61	31.48	0	0
5	8	25.36	26.43	5.42	7.55	2.55	3.55	24.71	33.11	0	0
6	12	25.88	28.66	7.98	9.35	3.33	5.24	24.86	34.12	0.19	0.30
7	24	24.96	28.79	9.46	9.89	4.24	5.57	24.97	34.80	0.79	2.05

HPLC: High-performance liquid chromatography

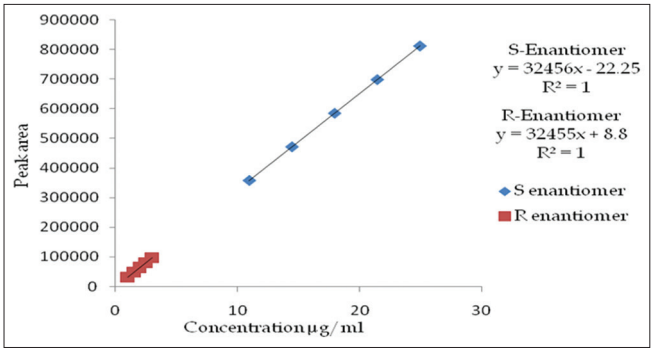

Fig. 6: Calibration curve of R and S rosiglitazone by high-performance liquid chromatography

Table 2: System suitability studies for the determination of R rosiglitazone and S rosiglitazone

S. No	Parameters	R Enantiomer	S Enantiomer
1.	Linearity range	3–7 µg/mL	9–21 µg/mL
2.	Regression equation y=mx+c	Y=32455x−8.8	Y=32456x−22.25
3.	Correlation coefficient	1.0	1.0
4.	Theoretical plate/meter	40074	45503
5.	Resolution factor	0.6	
6.	Asymmetric factor	1.02	1.01
7.	LOD (µg/mL)	0.596	0.596
8.	LOQ (µg/mL)	1.86	1.86

LOD: Limit of detection, LOQ: Limit of quantification

establishing that the developed method is accurate and reliable. Detection limit and quantification limit of enantiomer I and II were found to be 0.30 µg/ml and 0.90 µg/ml and 0.32 µg/ml and 0.97 µg/ ml, respectively. No marked changes in the chromatograms occurred on changing the instrument. Chromatographic conditions indicated that the developed method was rugged and robust. The column efficiency, resolution, and the peak asymmetry were calculated for the standard solutions. Signal-to-noise ratio of the 3 and 10 is generally considered as limit of detection and limit of quantification, respectively, Table 2.

CONCLUSION

A highly specific stability-indicating chiral HPLC method was developed for the 1st time to quantify the rosiglitazone enantiomers in the presence of degradation products. The enantioseparation was carried out by the use of cellulose-based chiral column. The method provides good sensitivity and excellent precision and reproducibility. The method was highly selective, where degradation products and coformulated compounds did not interfere. The proposed method was successfully applied in pharmaceutical preparations.

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AUTHORS' CONTRIBUTIONS

Dr. B. Gowramma has performed the work presented here. Dr. S. N. Meyyanathan has guided this project. Dr. B. Babu contributed to the data analysis and interpretation. Dr. N. Krishnaveni contributed to some part of the discussion.

CONFLICTS OF INTEREST

This study has no conflicts of interest to be declared.

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