March 2008 Volume 7 Issue 5

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ 7(5) 2008 [273-278]

A stability-indicating HPLC assay method for rosiglitazone maleate

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Received: 29th January, 2008 ; Accepted: 3rd February, 2008

ABSTRACT

This present paper deals with the development of a stability-indicating high performance liquid chromatographic (LC) method for the quantitative determination of Rosiglitazone maleate. The maleate salt of Rosiglitazone, an orally active thiazolidinedione with antidiabetic properties and potential antineoplastic activity. The chromatographic separation was achieved on a Phenomenex Luna 3µ C18 HPLC column using a mobile phase consists of a mixture of sodium dihydrogen orthophosphate buffer with sodium hexane sulfonate (Solvent A) and organic modifier acetonitrile and methanol (Solvent B). Forced degradation studies were performed on bulk sample of Rosiglitazone maleate using acid(1.0 N hydrochloric acid), base (0.05 N sodium hydroxide), oxidation (3.0% v/v hydrogen peroxide), thermal (80°C) and UV light (254nm). Degradation of the drug substance was observed in base hydrolysis and oxidation condition. Degradation product formed under base hydrolysis was found to be starting material used during the synthesis of Rosiglitazone maleate. The stressed samples were assayed using the developed LC method and found the mass balance was close to 99.5%, thus proves the stability-indicating power of the developed LC method. The developed LC method was validated with respect to linearity, accuracy, precision and robustness.

KEYWORDS

Forced degradation;
Validation;
Solution and mobile phase stability;
Rosiglitazone maleate;
High performance liquid chromatography (HPLC).

INTRODUCTION

The chemical name of Rosiglitazone maleate is propanamide, (±)-5-[4-[2-[N-Methyl-N-(2-pyridinyl) amino]ethoxy]benzyl] thiazolidine-2, 4-dione maleate. It belongs to an anti-diabetic drug (thiazolidinedione-type, also called "glitazones") Rosiglitazone maleate activates peroxisome proliferator-activated receptor gamma (PPAR-gamma), a ligand-activated transcription factor, thereby inducing cell differentiation and inhibiting cell growth and angiogenesis[1]. This agent also modulates the transcription of insulin-responsive genes, inhibits macrophage and monocyte activation, and stimulates adipocyte differentiation.

So far to our present knowledge no stability-indicating analytical method for Rosiglitazone maleate was available in the public domain. A sensitive and selective liquid chromatography-mass spectrometry method for the quantification of rosiglitazone in human plasma was published[2]. An investigation of racemisation of the enan-

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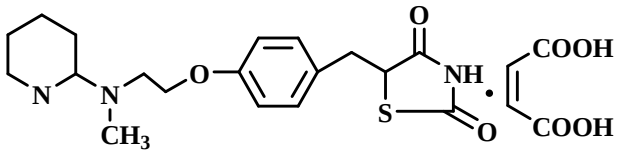
tiomers of glitazone drug compounds at different pH using chiral HPLC and chiral CE was also published[3]. It was felt necessary to develop a suitable stability-indicating HPLC Assay method for the quantitative determination of Rosiglitazone maleate. The present research work deals with the forced degradation of the drug substance under stress conditions like acid hydrolysis, base hydrolysis, oxidation, thermal and UV. The work also includes the validation of the developed stability-indicating LC Assay method.

Rosiglitazone maleate



(+)-5-[4-[2-[N-Methyl-N-(2-pyridinyl)amino] ethoxy benzyl] thiazolidine-2,4-dione maleate

Figure 1: Chemical structure of Rosiglitazone maleate

EXPERIMENTAL

Chemicals and reagents

Samples of rosiglitazone maleate figure 1 and its impurity namely dehydro Rosiglitazone maleate were received from Process Research Department of Active Pharmaceutical ingredients Operations of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile, methanol, Sodium dihydrogen orthophosphate, sodium n-hexane sulfonate and phosphoric acid were purchased from Merck, Schuchardt OHG, Germany. High pure water was prepared by using Millipore Milli Q plus purification system.

Instrumentation

The LC system used for method development, forced degradation studies and method validation was Shimadzu prominence model equipped with a photo diode array detector. The out put signal was monitored and processed using LC solutions software (Shimadzu) on hp computer (Digital Equipment Co).

Chromatographic conditions

The HPLC column used was Phenomenex Luna C18 (150×4.6mm, 3µ) using a mobile phase consists of solvent A (Dissolved 4.14 g of sodium dihydrogen orthophosphate one hydrate in 1000 mL of water and adjusted pH 3.0 with dilute ortho phosphoric acid and then added 1.88 g of sodium-n-hexane sulfonate) and solvent B(acetonitrile: methanol::10:13v/v). The flow rate of the mobile phase was kept at 0.8 mL/min with isocratic condition at mobile phase A: mobile phase B:70:30 The HPLC column was maintained at 40°C and the wavelength was monitored at a wavelength of 245nm. The injection volume was 20 µL. 80:20 mixtures of water and mobile phase B was used as a diluent.

Preparation of standard solutions

Working solution of 100 µg/mL was prepared for assay determination. Dehydro Rosiglitazone maleate stock solution at 500 µg/mL was also prepared in 20:80 (v/v) mixture of water and acetonitrile as a diluent.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Rosiglitazone maleate was carried out in the presence of its impurity namely Dehydro Rosiglitazone maleate.

Forced degradation studies were also performed for bulk drug to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254nm), thermal (80°C), acid (1.0 N HCl), base (0.05 N NaOH) and oxidation (3.0% H_2O_2 v/v) to evaluate the ability of the proposed method to separate Rosiglitazone maleate from its degradation products. For heat and light studies, study period was 10 days where as for acid, base and oxidation, it was 48 h. Peak purity test was carried out on the stressed samples of Rosiglitazone maleate by using PDA detector. Assay studies were carried out for stress samples against qualified reference standard and the mass balance (% assay+ % degradation) was calculated.

Method validation

Precision

Assay method precision was evaluated by carrying out six independent assays of test sample of Rosiglitazone maleate against qualified reference stan-

dard and calculated the % RSD of assay. The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory[4].

Linearity

Linearity test solutions for assay method were prepared from stock solution at six concentration levels from 25% to 150% of assay analyte concentration (25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL and 150 µg/mL). The peak area versus concentration data was performed by least-squares linear regression analysis. The calibration curve was drawn by plotting Rosiglitazone maleate average area for triplicate injections and the concentration expressed in percentage at each level.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels i.e. 50 µg/mL, 100 µg/mL and 150 µg/mL in bulk drug sample. The % recoveries were calculated.

Solution stability and mobile phase stability

The solution stability in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for two days. The same sample solutions were assayed for 6 h interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for six hours interval up to two days. Mobile phase prepared was kept constant during the study period. The % RSD of assay of Rosiglitazone maleate was calculated for

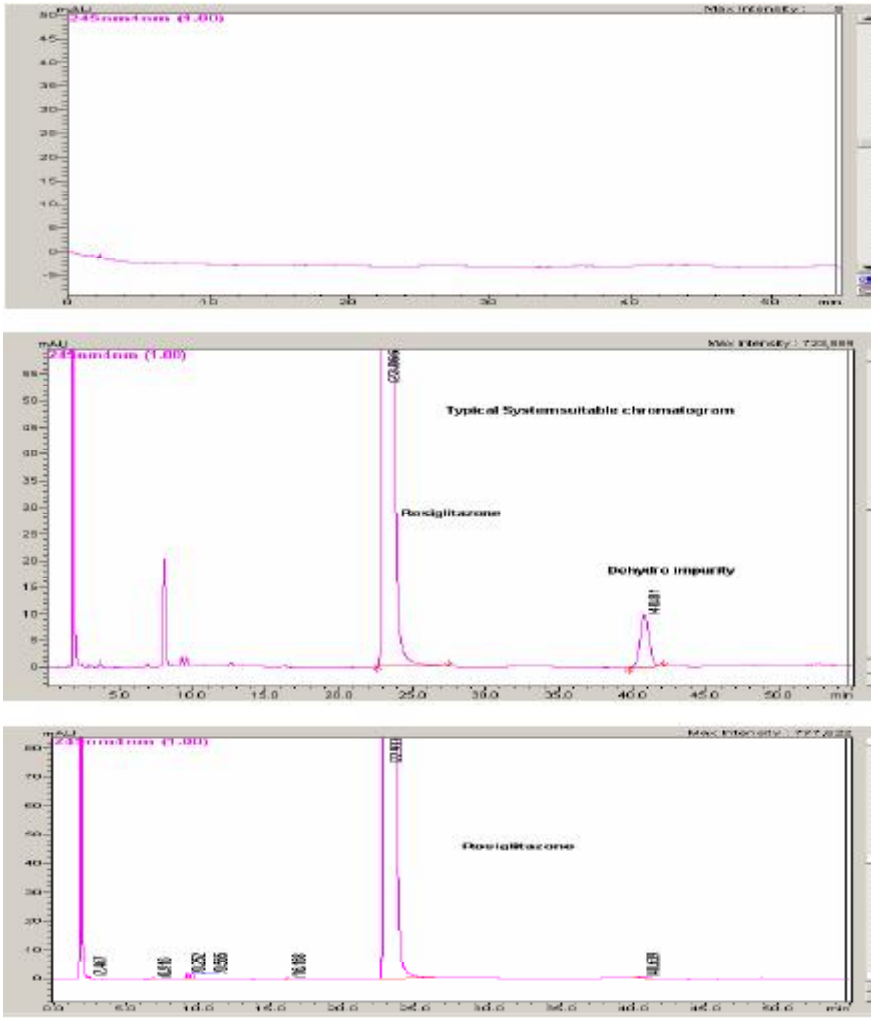


Figure 2: Typical HPLC chromatogram of system suitability

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the study period during mobile phase and solution stability experiments.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Dehydro Rosiglitazone maleate impurity was the potential impurity present in bulk samples produced by Dr.Reddy's laboratories. The main target of the chromatographic method is to get the separation of Dehydro Rosiglitazone maleate, Rosiglitazone maleate and also degradants formed during forced study. Using different stationary phases like C18, C8 , CN and different mobile phases containing buffers like phosphate, sulphate, acetate with different pH (3-7) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. The chromatographic column used was Phenomenex Luna C18 (150×4.6mm, 3µ) column using a mobile phase consists of a mixture of solvents A and solvent B. The flow rate of the mobile phase was 0.8 mL/min. The HPLC isocratic condition was kept as mobile phase A: mobile phase B: 70:30. The column was maintained at 40°C and the wavelength was monitored at a wavelength of 245 nm. In the optimized conditions, Rosiglitazone maleate, Dehydro Rosiglitazone maleate impurity and all the degradant products were well separated and the developed isocratic LC method was found to be specific for Rosiglitazone maleate and its impurities. Typical HPLC blank and the System suitability chromatograms were presented in figure 2.

TABLE 1: Summary of forced degradation results

Degradation condition	Time	Assay (%w/w, anhydrous basis)	Mass balance (% assay+% degradation products)	Remarks
Control sample	-	99.7	-	-
Thermal at 80°C	10 days	98.9	99.8	No significant degradation observed
Acid hydrolysis 1.0 N HCl	48h	99.7	99.6	No significant degradation observed
Base hydrolysis, 0.05 N NaOH	3h	77.1	99.8	Significant degradation observed
Oxidation by H_2O_2 (3.0 % v/v)	48h	83.7	99.7	Significant degradation observed
UV at 254 nm	10 days	99.7	99.6	No significant degradation observed

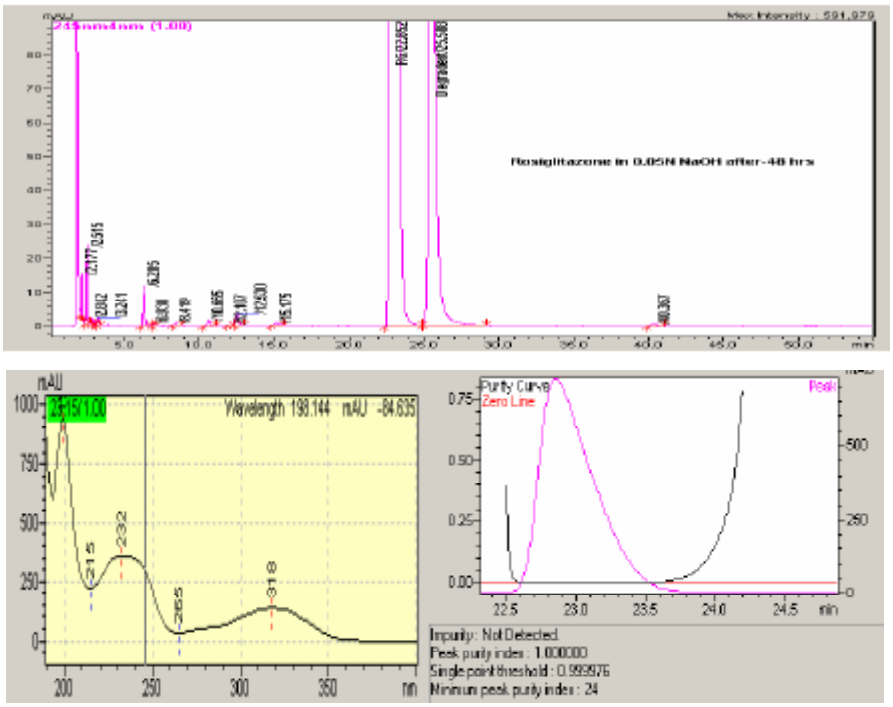

Figure 2: Typical HPLC peak purity chromatogram of Rosiglitazone malete in 0.05 N NaOH.

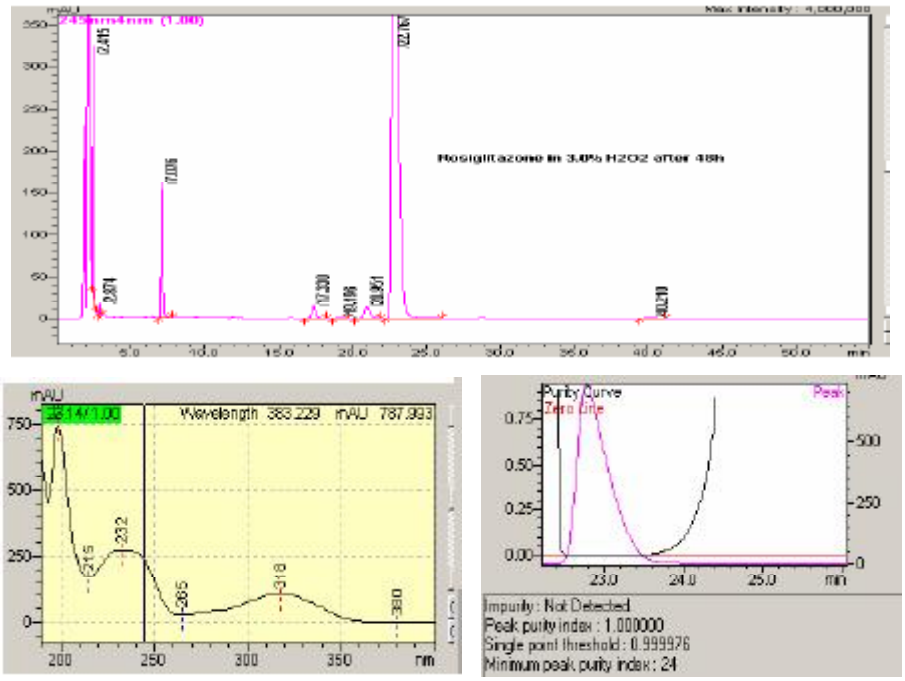

Figure 3: Typical HPLC peak purity chromatogram of Rosiglitazone malete in 3.0% H_2O_2

Results of forced degradation studies

Degradation was not observed in Rosiglitazone maleate samples during stress conditions like UV light[5], thermal, and acid hydrolysis. The degradation of drug substance was observed in base hydrolysis and under oxidative stress (Figures 3 and 4). Rosiglitazone maleate was degraded into unknown impurity during base hydrolysis (in 0.05 N NaOH after 48 h treatment). Peak purity test results confirm that the Rosiglitazone maleate peak is homogeneous and pure in all the analyzed stressed samples of Rosiglitazone maleate. The mass balance of stressed samples was close to 99.5% (TABLE 1).

Precision

The % RSD of assay of Rosiglitazone maleate during assay method precision study was well within 1% and the % RSD of assay results obtained during intermediate precision study was within 1.0% thus confirming good precision of the method.

Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 25 µg/mL to 150 µg/mL and the correlation coefficient obtained was greater than 0.999. Linearity was checked for assay method over the same concentration range for three consecutive days.The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Accuracy

The percentage recovery of Rosiglitazone maleate in bulk drug samples was ranged from 98.3 to 101.4% w/w.These results shows that the developed LC method was accurate.

Robustness

In all the deliberate varied chromatographic conditions (like flow rate and column temperature) no significant change in assay value was observed, which confirms the robustness of the developed LC method.

Solution stability and mobile phase stability

The % RSD of assay of Rosiglitazone maleate during solution stability and mobile phase stability experiments was within 1.0% RSD. No significant change was observed in assay content of Rosiglitazone maleate during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay are stable up to 48 h.

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CONCLUSIONS

The RP-LC method developed for quantitative determination of Rosiglitazone maleate is precise, accurate, linear, robust and selective. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability-indicating and can be used for assessing the stability of bulk samples of Rosiglitazone maleate.

ACKNOWLEDGMENTS

The authors wish to thank the management of Dr. Reddy's group for supporting this work. Authors wish to acknowledge the Process research group for providing the samples for our research. We would also like to thank colleagues in separation science division of Analytical Research of Active Pharmaceutical Ingredients for their co-operation in carrying out this work.

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