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RP-HPLC Method for the Determination of Rosiglitazone in presence of its Degradation Products in Bulk Drugs

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ABSTRACT

This study describes an isocratic RP-LC method that uses a water rich mobile phase for the estimation of rosiglitazone in presence of its degradation products generated from forced decomposition studies. The separation was achieved with a C_{18} column using mobile phase comprising of water: methanol: ortho phosphoric acid (80: 20: 0.2, v/v), the pH of which was adjusted to 4.5 with the help of liquid ammonia. The flow rate was kept at 1 ml/min and analyte was screened with UV detector at 230 nm. The retention time for rosiglitazone was found to be 4.97 minutes. The drug was found to degrade extensively in oxidative degradation and mild degradation in acidic degradation while it was found stable in alkaline stress. The developed method was precise and sensitive and could be applied successfully to determine rosiglitazone in the presence of its degradation products.

Key words: Rosiglitazone Maleate, HPLC, Degradation product.

INTRODUCTION

Rosiglitazone maleate (ROS) having molecular formula 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl] methyl]-2,4-thiazolidinedione; is one of the newly available member of the thiazolidinedione family that acts primarily by reducing insulin resistance [1,2]. The structure of rosiglitazone is shown in figure1.

There are various methods reported for estimation of ROS in tablets [3,4], in human plasma [5,6,7] and in urine [8]. The methods are also available for the simultaneous estimation of ROS in combination with other antidiabetics [9-14]. The evaluation of quality of drug substance requires complete understanding on the chemistry of drug molecule, its potential process and degradation related impurities.

The aim of present work was to develop an accurate, selective, precise and stability indicating RP-HPLC method for the determination of ROS in the presence of its degradation products.

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The LC system (Analytical, India) consisted of a solvent delivery module (ALC), analytical manual injector 2010 fitted with a 20 µL injection loop and a UV detector (ASPD). The column used was Grace Smart C_{18} 5micron {250 mm x 4.6 mm i.d.}. The mobile phase was prepared by mixing methanol: water: ortho phosphoric acid in the ratio 80: 20: 0.2, v/v; and the final pH was adjusted to 4.5 with liquid ammonia. The flow rate was kept at 1 ml/min. The detection wavelength was set to 230 nm. Operation and data acquisition was performed using Analchrom and analysis was performed using Clarity. Mobile phase was degassed by ultrasonication (Toshcon, India).

These chromatographic conditions were developed by following a series of experiments in an effort to elute rosiglitazone maleate at a retention time that is suitable for analysis with adequate resolution from its degradation products.

Reagents

Reference standard of rosiglitazone maleate (ROS) was procured from Zydus Research Center (Ahmedabad, Gujarat, India). Methanol was of LC grade (Rankem, Delhi); while ortho-phosphoric acid (Rankem, Delhi) and liquid ammonia (S. D. fine Chem., Mumbai) were of analytical reagent grade. Deionised water was prepared using a triple stage water purifier system; that was further filtered through 0.2 micron filter.

Preparation of Calibration Standards and Quality Control Samples

Standard stock solution (1000 µg/ml) of ROS was prepared in methanol and was kept at 4 ^{0}C. Further dilutions were prepared in mobile phase to obtain working standards in a concentration range of 5-30 µg/ml.

Forced Degradation Studies (Stress testing)

The drug concentration for all stress studies was taken 1 mg/ml. The bulk drug was subjected to alkaline studies by adding 1 ml of the 1N NaOH for 12 hours and neutralized with 1 ml of 1N HCl. Similarly, the acidic study was performed by adding 1 ml of the 1N HCl for 12 hours and neutralized with 1 ml of 1N NaOH. Oxidation study was performed on bulk drug by adding 1 ml of 5% H_2O_2 for 12 hours. All samples were taken in different 10 ml volumetric flasks and dissolved in mobile phase. Final assay drug concentration of 15 µg/ml was made up with mobile phase and injected in the chromatographic system. All stressed samples were analyzed by developed HPLC method [15].

RESULTS AND DISCUSSION

Method Validation

The proposed method was validated with respect to stability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision according to the ICH Guidelines [16,17].

Calibration and linearity for Validation

A standard curve of six points was constructed by plotting peak area versus concentration of ROS as shown in figure 2. Standard dilutions of the drug were prepared in the concentration range of 5-30 µg/ml. A calibration curve of the drug was constructed and linearity was assessed by least square regression analysis. The correlation coefficient (r^2) was determined which should be 0.999 or better. The acceptance criteria of standard concentration were ±15% deviation from the nominal value except LLOQ, which was set as ±20%.

Precision and Accuracy

Precision and accuracy were determined by the analysis of three concentrations chosen from the high, medium and low range of the standard curves for the selected drug (5, 15 and 25 µg/ml). Triplicates of each samples (n=9) were analyzed on day 1 to determine intra-day precision and accuracy. Inter-day precision and accuracy was determined by triplicate samples of these concentrations on day 1 to 6. Mean, standard deviation and relative standard deviation were calculated from these concentration values and used in the estimation of intra- and inter-day precision. Accuracy (bias) is expressed as the percent difference between calculated mean concentrations relative to the nominal concentration. A precision (%CV) ≤5% and an accuracy (%bias) ≤ ±15% are acceptable. The RSD values of intra- and inter-day studies varied from 0.613 to 1.948% which showed that the precision of method was satisfactory (Table 1).

The data show good precision of the system with a RSD ≤5% (Table 2). Method precision was determined from the results from six independent determinations at 100% of the sample concentrations of ROS.

Stability Studies

Stability of the standard solutions of ROS was evaluated under different storage conditions. The long-term stability was assessed after storage of stock solutions at 4 ^{0}C for 6 days. The stock solution of ROS was analysed on 3rd and 6th day and the response were compared with the response obtained from the analysis of stock solutions on 1st day. The results showed that the retention time and peak area were almost unchanged (RSD % < 5) and that no significant degradation was observed within the given period, indicating the solutions are stable for at least 6 days.

Specificity

Specificity, described as the ability of a method to discriminate the analyte from all potential interfering substances, was evaluated by preparing the analytical placebo and it was confirmed that there were no peaks obtained at the same retention time of the drug. Also, the extraneous peak, if obtained, was well resolved from the peak of the analyte. A solution of an analytical placebo (containing tablet excipients namely lactose, magnesium stearate, dextrose, carboxymethyl cellulose and talc, except the analyte) was prepared according to the sample preparation procedure and injected. The representative chromatogram showed no other peaks at the retention time of the analyte, which confirmed the specificity of the method.

Linearity

The linearity was determined by plotting the graph between peak area and concentration of the drug. The peak area responses for six concentrations were determined. The concentrations used for analysis were in the range of 5-30 µg/ml for ROS. The linearity curve was defined by the following equation: y = 33.6152x - 7.6387 and coefficient of correlation was found to be r^2 = 0.9999. The y is the peak area and x is the concentration expressed in µg/ml.

LOD and LOQ

For determining the limit of detection (LOD) and limit of quantitation (LOQ) the method based on the residual standard deviation of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of 5-30 µg/ml. The LOD and LOQ were found to be 0.1 and 0.31 µg/ml, respectively.

Analysis of stressed samples

The ICH stability guideline Q1A (R2) defines stress testing for new drug substances and drug products, to elucidate the intrinsic stability of the drug substances and drug products [18]. The drug was found to degrade extensively in oxidative degradation and mild degradation in acidic degradation while it was found stable in alkaline stress (Figure 2-4). Degradation percent assay values are given in (Table 3).

Tables

Table 1. Intra-day and Inter-day accuracy and precision data of Rosiglitazone Maleate

Spiked Conc. (µg/ml)	Intra-day			Inter-day		
	Found[a] (µg/ml)	Precision %CV	Accuracy[b] %Bias	Found[a] (µg/ml)	Precision %CV	Accuracy[b] %Bias
5	4.985 ± 0.031	0.613	-0.3	4.93 ± 0.053	1.079	-1.4
15	15.086 ± 0.121	0.803	+0.573	14.664 ± 0.261	1.779	-2.24
25	24.578 ± 0.307	1.251	-1.688	24.404 ± 0.476	1.948	-2.384

a - Mean ± standard deviation, n=3

b - Bias % = [(found-spiked)/spiked] x 100

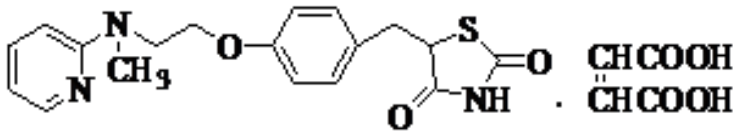
Table 2. System precision data for Rosiglitazone Maleate

Factors	Result	
	Mean ± SD	% CV
Retention time (min)	4.97 ± 0.058	1.16
Capacity factor		
Peak asymmetry		
Resolution*	-	

* = *Resolution from the nearest peak resulted due to degradation*

Table 3. Degradation % assay value

Stress Conditions	% assay of drug after exposed to stress condition
Alkaline – 12 h	99.13
Acidic – 12 h	96.43
Oxidative – 12 h	82.02



Rosiglitazone Maleate

Figure 1. Chemical Structure of Rosiglitazone Maleate

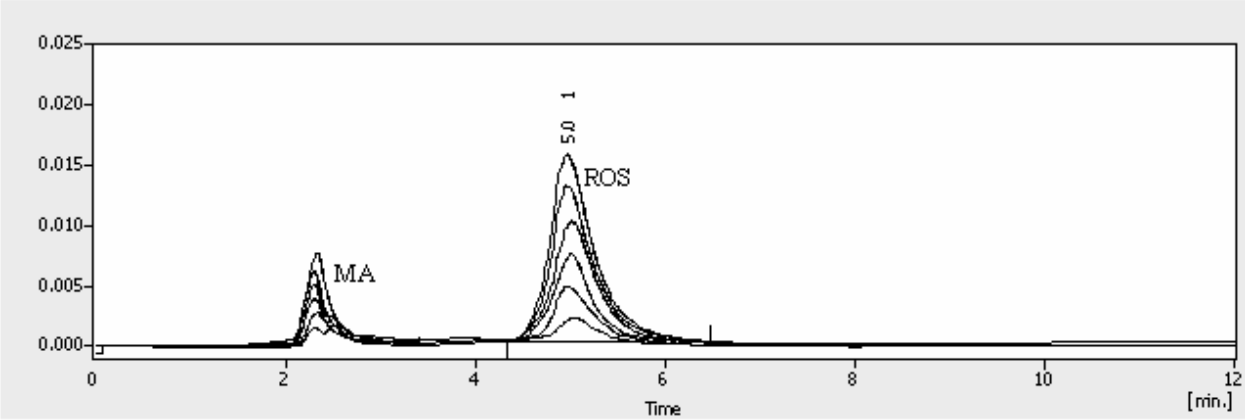


Figure 2. Representative chromatogram of Rosiglitazone for calibration curve (5-30 µg/ml); where ROS is peak of rosiglitazone and MA is peak of maleic acid

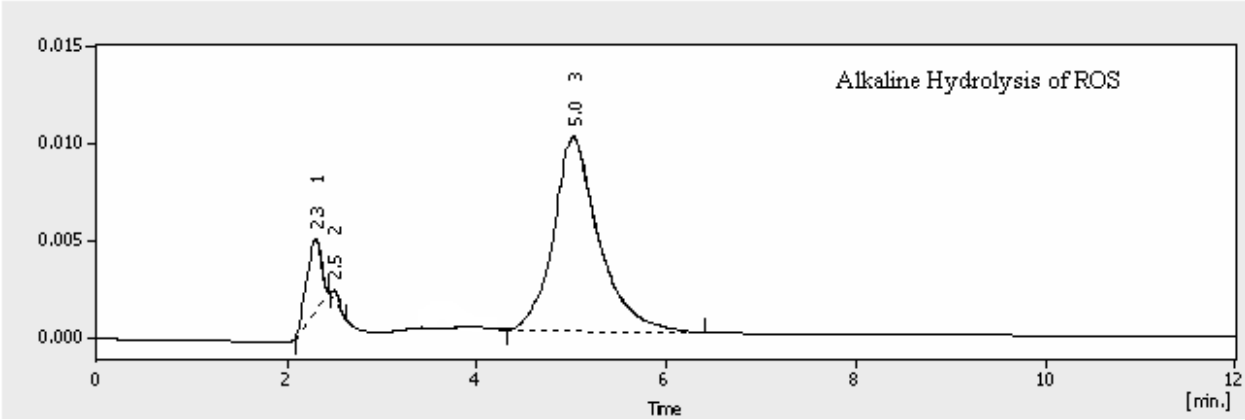


Figure 3. Representative chromatogram of ROS under Alkaline Hydrolysis for 24 hr.

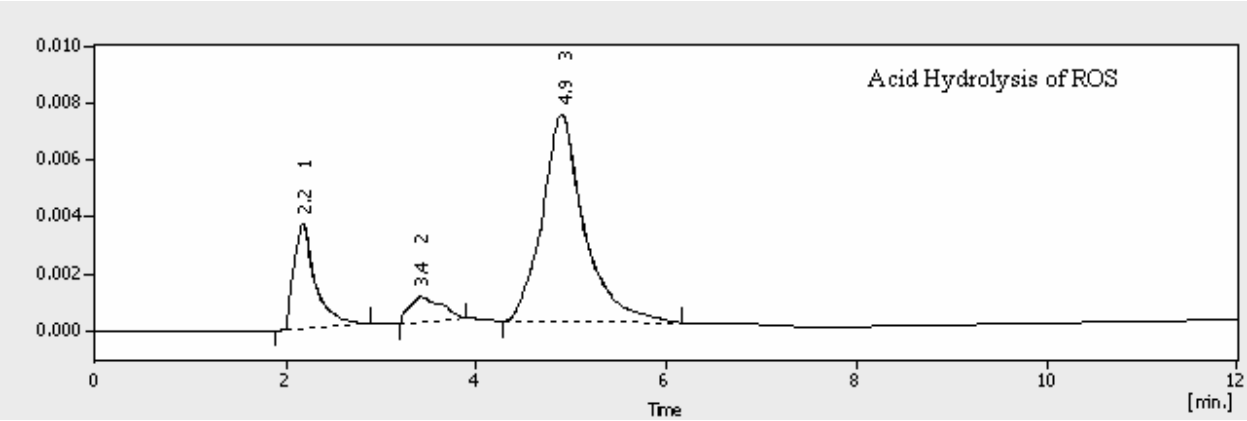


Figure 4. Representative chromatogram of ROS under Acid Hydrolysis for 24 hr.

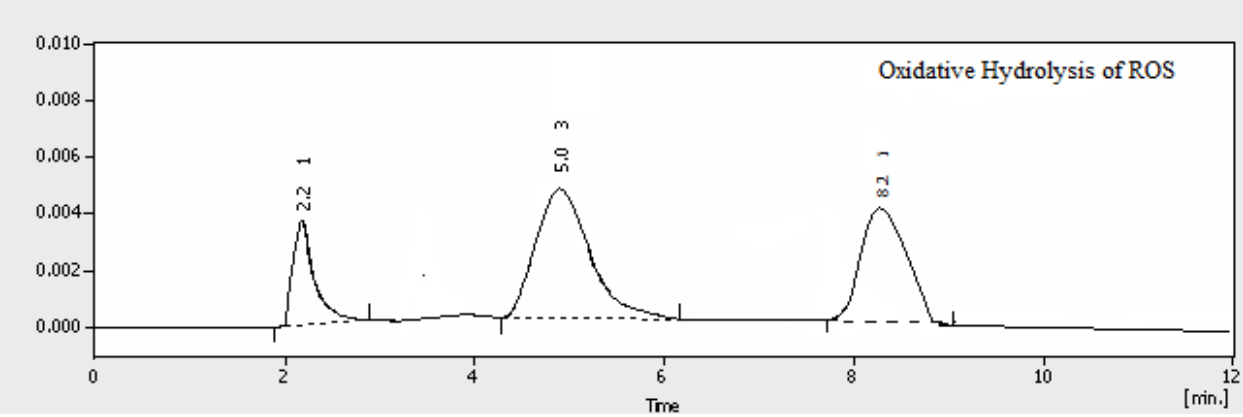


Figure 5. Representative chromatogram of ROS under Oxidative Hydrolysis for 24 hr.

CONCLUSION

In this work, a simple, fast and reliable LC method was developed for the determination of ROS in presence of its degradation products in bulk drugs. Composition of mobile phase as well as pH value of mobile phase has played important role in method development. Several mobile phases were prepared in different ratios were tried at different pH. Finally, good peak shape and optimum resolution between ROS and its degradation products was observed using the mobile phase mentioned in the chromatographic condition. Forced degradation studies revealed that possible degradation products do not interfere with the determination of rosiglitazone. The developed method after being completely validated showed satisfactory data for all method validation parameters. The method was found to be accurate and precise as it exhibited low %bias and %RSD, respectively. Moreover, the lower solvent consumption along with the shorter analytical run time of less than 5.5 min leads to cost effective chromatographic method.

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