

DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR ESTIMATION OF ROSIGLITAZONE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated of rosiglitazone in bulk and tablet dosage form. Chromatographic analysis was performed on a C₁₈ column (250x 4.6 mm, 5 μ m) with a mixture of Ammonium dihydrogen phosphate buffer (pH 4.5): Acetonitrile in the ratio 65:35 as mobile phase, at a flow rate of 1.0 mL min⁻¹. UV detection was performed at 230 nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The retention times of rosiglitazone was found to be 7.19 \pm 0.145 respectively. Linearity was observed in concentration ranges of 12–70 μ g mL⁻¹. The limit of detection was 0.725 and the quantification limit was 2.41 μ g/ml. The accuracy of the proposed method was determined by recovery studies and found to be 98.26% to 101.37%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of rosiglitazone and in bulk and tablet dosage form.

Keywords: Diclofenac; NSAID; Cream; Topical; Stability

1. Introduction

Rosiglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4-Dione. Rosiglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamma receptors have acts primarily enhances tissue sensitivity to insulin. A literature survey reveals that various analytical methods like rosiglitazone by HPLC and MECK², Simple HPLC method for the determination of rosiglitazone in human plasma^{3,4}, Simultaneous HPLC estimation of metformin in combination with rosiglitazone⁵, rosiglitazone with gliclazide in tablet⁶, rosiglitazone and gemfibrozil in human plasma⁷. Simultaneous LC-UV estimation of rosiglitazone and glimepiride in plasma⁸, rosiglitazone and glimepiride in human plasma⁹. Simultaneous LC-ESI-MS method for estimation of rosiglitazone and N-desmethyl rosiglitazone in human plasma¹⁰.

But these methods are sophisticated, expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproducible HPLC method for the estimation of rosiglitazone in bulk and its formulation.

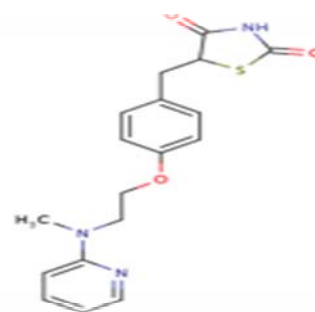


Fig.: Chemical structure of Rosiglitazone

2. Experimental

2.1. Materials and Methods: Pure standard of rosiglitazone (Assigned purity 99.98%) was obtained as a gift sample from Micro labs Pvt. Ltd, Badi, India. The gift samples

were used as standard without further purification. HPLC grade water, methanol (Qualigens), ammonium dihydrogen phosphate, glacial acetic acid and ammonia (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. Commercial pharmaceutical preparation (Avandia) which was claimed to contain 4mg of rosiglitazone is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

2.2. Instrumentation and chromatographic conditions: High performance liquid chromatography, Shimadzu pump LC-10AT VP equipped with universal injector (Hamilton 25 μ L) SPD10A, UV-VIS detector SPD10A-10A VP (Shimadzu) was used. Isocratic elution of mobile phase comprising of Ammonium dihydrogen phosphate buffer 65% (pH 4.5) Acetonitrile 35% (pH 4.5) flow rate of 1.0 ml min⁻¹ was performed on C₁₈ column (250x 4.6 mm, 5 μ m). The effluent was detected at 230 nm. The retention times of rosiglitazone were 7.19 \pm 0.145min. The column temperature was maintained at ambient and the volume of injection was 20 μ l. Prior to injection of analyte, the column was equilibrated for 30- 40 min with mobile phase.

2.3. Preparation of mobile phase: The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of Ammonium dihydrogen phosphate buffer 65% (pH 4.5) Acetonitrile 35% [(Solvent A), Ammonium dihydrogen Phosphate Buffer: Dissolve 2.003 gm of ammonium dihydrogen phosphate (0.02 M) in 1000 ml of water, adjust the pH to 4.5 with ammonia or glacial acetic acid, (solvent B) Acetonitrile]. The contents of the mobile phase were mixed in the ratio of ammonium dihydrogen phosphate buffer: acetonitrile (65:35) and phase were filtered before use through a 0.45 μ m membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

2.4. Standard solution: Standard stock solutions 1 mg mL⁻¹ of rosiglitazone was prepared in methanol and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain

mixture of rosiglitazone in over the linearity range from 20 –70 μ g/ml.

2.5. Assay in formulation: Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogenous powder and a quantity equivalent to one tablet were weighed in to a 100 ml volumetric flask, dissolve in methanol, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 ml volumetric flask through 0.45 μ m membrane filter. The residue was washed 3 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. Further add mobile phase to obtain an expected concentration of 10 μ g/ml. All determinations were conducted in triplicate.

3. Results and Discussions

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatograms of rosiglitazone are shown in (Fig No.1). There was clear resolution between rosiglitazone with retention time of 7.19 \pm 0.145 minutes respectively.

3.1. Linearity: Linearity was observed in concentration ranges of 12–70 μ g/ mL. The response was determined to be linear over the range of 20 μ g/ml to 70 μ g/ml (20, 30, 40, 50, 60, and 70). The solutions were injected into HPLC system. Each of the concentration was injected in triplicate to get reproducible response. The run time was 13 min and the peak areas were measured. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation. (Fig 2& 3 calibration curve)

$$Y_{\text{Rosiglitazone}} = 511985x + 1060943$$

Coefficient of correlation (r^2) value = 0.9998

3.2. Accuracy: The accuracy is the closeness of the measured value to the true value for the

sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 1 ml, 3ml and 5 ml of sample drug from rosiglitazone solution of 100µg/ml were pipetted into each of three volumetric flasks. To this 1 ml of standard drug solution of 100µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. 20 µl of each solution was injected and chromatograms were recorded. The range was found between 98.26 to 101.37 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. (Table No.2)

3.3. Limit of detection and quantification:

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$L.O.D. = 3.3(SD/S)$ Where, SD = Standard deviation of the response

$L.O.Q. = 10(SD/S)$ S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The LOD was found to be 0.725 µg/ml and LOQ was found to be 2.41 µg/ml and for rosiglitazone respectively which represents that sensitivity of the method is high.

3.4 Precision: Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 40 µg/ml for rosiglitazone (n =6) showed a RSD of 0.337% for rosiglitazone. It was

concluded that the analytical technique showed good repeatability. (Table No.3)

3.5. Reproducibility and Ruggedness: The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates. (Table No.4)

3.6. Robustness: The robustness of the method was determined by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

3.7. Specificity: The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective.

It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of rosiglitazone is very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig No.4)

3.8. System Suitability: A binary solution of 70 µg mL⁻¹ of rosiglitazone (in triplicate) was prepared and same was injected, then the system suitability parameters were calculated from the following chromatogram. (Table No. 5)

Conclusion

The proposed RP-HPLC method is found to be simple, accurate, precise, linear, and specific, and, for quantitative estimation of rosiglitazone in bulk and its tablet

formulation. Therefore, the HPLC method described here can be used for routine analysis in bulk and its tablet formulation.

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Table No.1: For Peak Area of Rosiglitazone

Conc. In $\mu\text{g/mL}$	20	30	40	50	60	70
Replicate 1	11210714	16437253	21543371	26617388	31692012	36967508
Replicate 2	11228134	16527580	21642513	26518298	31256978	37025146
Replicate 3	11202714	16655058	21607365	26845881	31741253	37085964
Avg	11213854	16539964	21597750	26660522	31563414	37026206
SD	12997.65	109429.3	50265.55	167997.3	266521.3	59235.11
RSD	0.115907	0.661605	0.232735	0.630135	0.844399	0.159982

Table No.2 Result of recovery studies

S.No	Conc. taken in ($\mu\text{g/ml}$)	Std addition in ($\mu\text{g/ml}$)	Total Conc. found in ($\mu\text{g/ml}$)*	% recover
1	10	10	20.27	101.373 \pm 1.519
2	10	30	39.308	98.269 \pm 0.100
3	10	50	60.64	101.088 \pm 0.457
Mean \pm SD	100.240 \pm 1.72			

Table No.3 Result of repeatability analysis

S.No.	Conc. (µg/ml)	Peak Area (µV*sec)	Mean ± SD	% RSD
1	ROSIGLITAZONE 40(µg/ml)	21151753	211321191 ±71311.03	0.337452
2		21245204		
3		21092287		
4		21031368		
5		21120761		
6		21151773		

Table No.4 Results of reproducibility

Parameter	Result observed
	ROSI
Average Percentage Recovery	99.20%
SD between set of analysis on same date	0.432
SD between set of analysis on different date	1.06
RSD between set of analysis on same date	0.442%
RSD between set of analysis on different date	1.06%

Table No 5. Results of system suitability parameters

Parameters	Data obtained
	ROSI
Number of theoretical plates	4153
Symmetry factor/ Tailing factor	1.21

Fig No. 1. Typical chromatogram showing rosigitazone

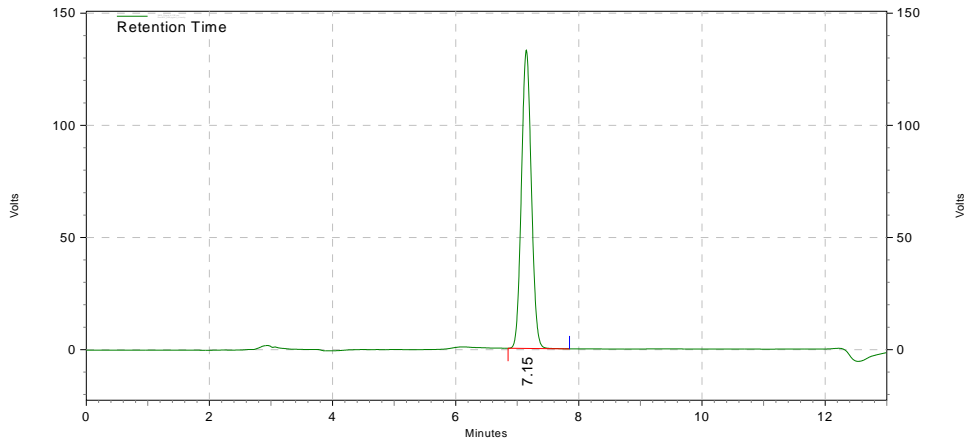


Figure 2: Calibration curve for Rosigitazone

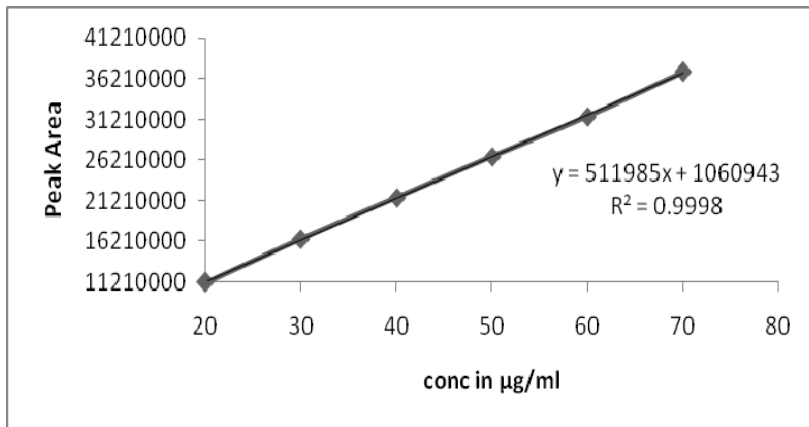


Figure No.3. Chromatogram showing Specificity

