Asian Journal of Pharmaceutical Analysis
and
Medicinal Chemistry

Journal home page: www.ajpamc.com

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE ESTIMATION OF ROSIGLITAZONE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
A simple, accurate, precise, and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of rosiglitazone in tablet dosage forms. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of Chloroform:Methanol (7:2) v/v. The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 200 to 2000 ng mL^{-1} with regression coefficient of 0.9992 and Rf value 0.53±0.01. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 98.67 to 99.11 %. The proposed method is applicable to routine analysis of rosiglitazone in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

KEYWORDS
Rosiglitazone, HPTLC and ICH guidelines.

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INTRODUCTION
Rosiglitazone (Figure No.1) 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[1]. 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 MECK[2], Simple HPLC method for the determination of rosiglitazone in human plasma [3, 4], Simultaneous HPLC estimation of metformin in combination with rosiglitazone[5], rosiglitazone with gliclazide in tablet[6], rosiglitazone and gemfibrozil in human plasma [7]. Simultaneous LC-UV estimation of rosigliazone and glimepiride in plasma[8], rosigliazone and glimepiride in human plasma[9]. Simultaneous LC-ESI-MS method for estimation of rosigliazone and N-desmethyl rosiglitazone in human plasma[10].

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.

EXPERIMENTAL DETAILS
Materials
Pure standard of Rosiglitazone (Assigned purity 99.98%) was obtained Micro labs Pvt. Ltd, Badi, India as a gift sample. The gift sample was used as standard without further purification. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Qualigens. 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.

Equipment
The instrument used in the present study was Camag Linnomat V-semiautomatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner 3, Cagmag Twin trough chamber of appropriate size (20X 20), Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH) were used throughout the experiment. Cagmag Wincats software was used for acquisition, evaluation and storage of chromatographic data.

Preparation of Standard Solution
A stock solution of drug was prepared by dissolving 100 mg of Pure Rosiglitazone in a 100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 200-2000ng/ml.

Procedure for Sample Solution
(From Formulation)
To determine the content of the drug in a solid dosage form, 20 tablet of rosiglitazone (4 mg) were accurately weighed, their average weight was calculated. Powder equivalent to 4 mg of the drug (content of one tablet) was dissolved in sufficient amount of methanol to dissolve the drug, sonicated for about 15 min. and then filtered into a 100 ml volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of methanol, and then the volume was completed to 100 ml with the same solvent. Make further dilutions with methanol to obtain a stock solution of 10µg/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up sufficient volume with the methanol to give an expected concentration of 1 µg/ml.

Prewashing of TLC plates
HPTLC was performed on 20 cm × 10 cm precoated silica gel 60 F 254 TLC plates. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 min. at 110 C, with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

RESULTS AND DISCUSSION

A methanolic solution of rosiglitazone (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 1µg/ml. Different concentrations of rosiglitazone in a concentration range of 200- 2000ng/ml were applied on plates as 8 mm bands, 8 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100 µl Hamilton syringe. A methanol blank was applied to parallel track. The mobile phase, Chloroform: Methanol (7:2) v/v was poured into the twin trough glass chamber and the glass chamber left to equilibrate for 10 min at 25 ± 2^0 C. After that the plate was placed in Camag twin trough glass chamber. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 254 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of rosiglitazone at various concentrations. This method was followed for all quantitative analysis. The Wincats software was used for data acquisition and processing of the plate. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

VALIDATION OF ANALYTICAL METHOD[11]

Validation of an analytical method is process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Typical analytical parameters used in validation area[11]

Linearity

A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 200-2000ng/ml. (Figure No.2 and Figure No.3) Coefficient of correlation (r2) should be greater than 0.998.

Accuracy

The accuracy is the closeness of the measured value to the true value of the sample. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.2 ml, 0.3ml and 0.7 ml of sample drug (Rosiglitazone) solution of 10µg/ml were pipetted into each of three volumetric flasks. To this 0.1 ml of standard drug (Rosiglitazone) solution of 10µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. The range of recovery studies were found between 98.34 to 99.13 %. 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.1).

Precision

Precision is measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The repeatability study which was conducted on the solution having the concentration of about 800 ng/ml for rosiglitazone the chromatograms were recorded to determine mean standard deviation and relative standard deviation. (Table No.2) From the above analytical data it is observed that RSD for the assay is 1.967 which indicates that the method is precise and reproducible.

Specificity

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drugs was spiked and observe the chromatogram (Figure No.4). The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for rosiglitazone.

Limits of Detection and Quantification

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The lower limit of detection for Rosiglitazone is 46.57 ng/ml in reference material and formulation. Limit of Quantification (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOQ values were found to be 155.23ng/ml for raw material and formulations.

Range

The specific range derived from the linearity studies. The range was calculated from the linearity graph. From the lower to higher concentration between which the response is linear, accurate and precise. Acceptance criteria: RSD < 2.0. The range for rosiglitazone was found to be 200-2000 ng/ml.

Solution Stability

The solution stability of the standard and sample prepared in methanol was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for period of more than 48 hours.

Table No.1: Result of recovery studies

S.No	Conc.taken in ng/ml (A)	Std addition in ng/ml (B)	Total drug conc. in ng/ml (A+B)	Peak Area	% Recovery ± SD	Average	% RSD
1	100	100	200	1813.2	98.84±0.629		
2	100	300	400	2366.16	98.61±0.521	98.85	0.253
3	100	700	800	3392.3	99.11±0.981		

Table No. 2: Precision of Rosiglitazone

S.No	Area Response	Average ± SD	% RSD
1	3253.3		
2	3379.5		
3	3368.8	3303.24±64.97	1.967
4	3262.8		
5	3251.8		
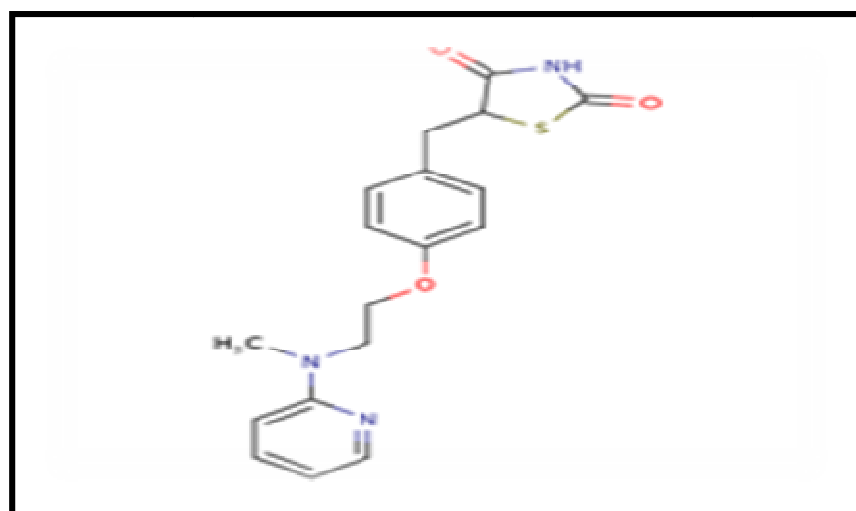
Figure No.1: Chemical structure of Rosiglitazone

Figure No.2: Linearity

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area
1	0.51	0.1	0.54	339.7	100.00	0.57	2.5	6268.8
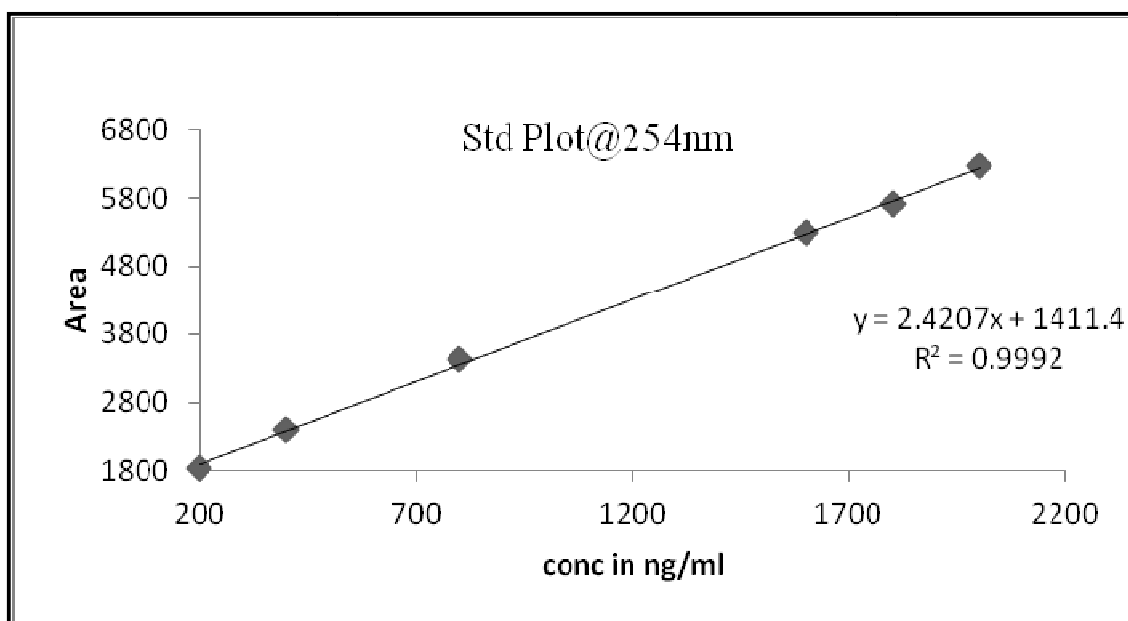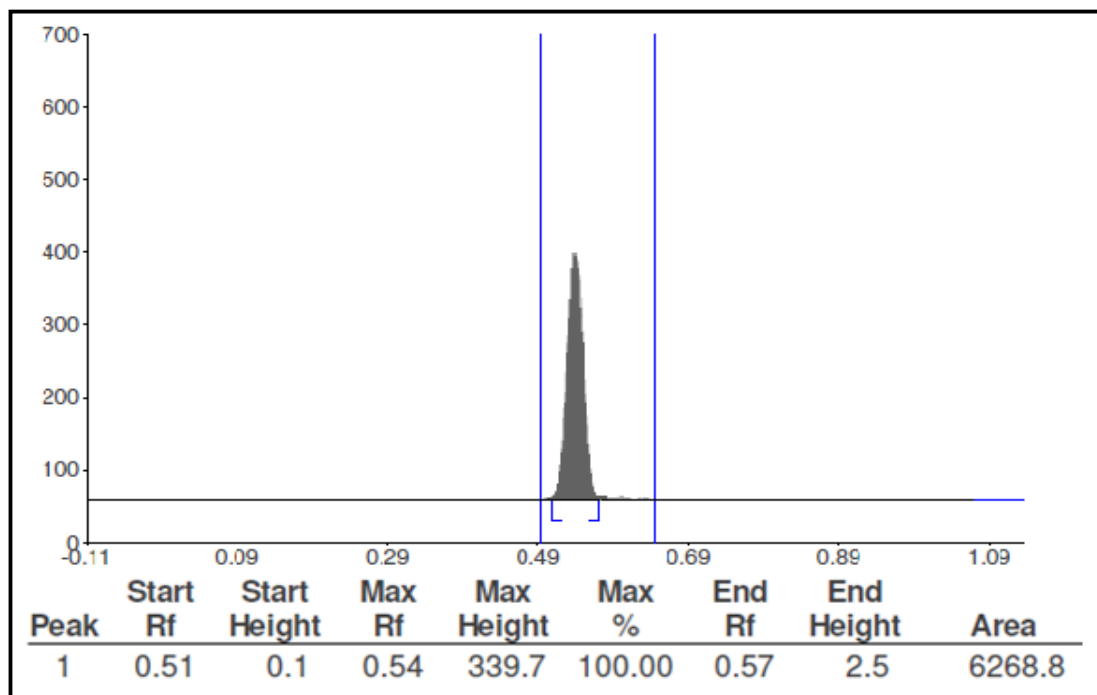
Figure No.3: Peak Area for Rosiglitazone

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.50	1.9	0.54	304.8	100.00	0.57	0.3	5287.5	100.00

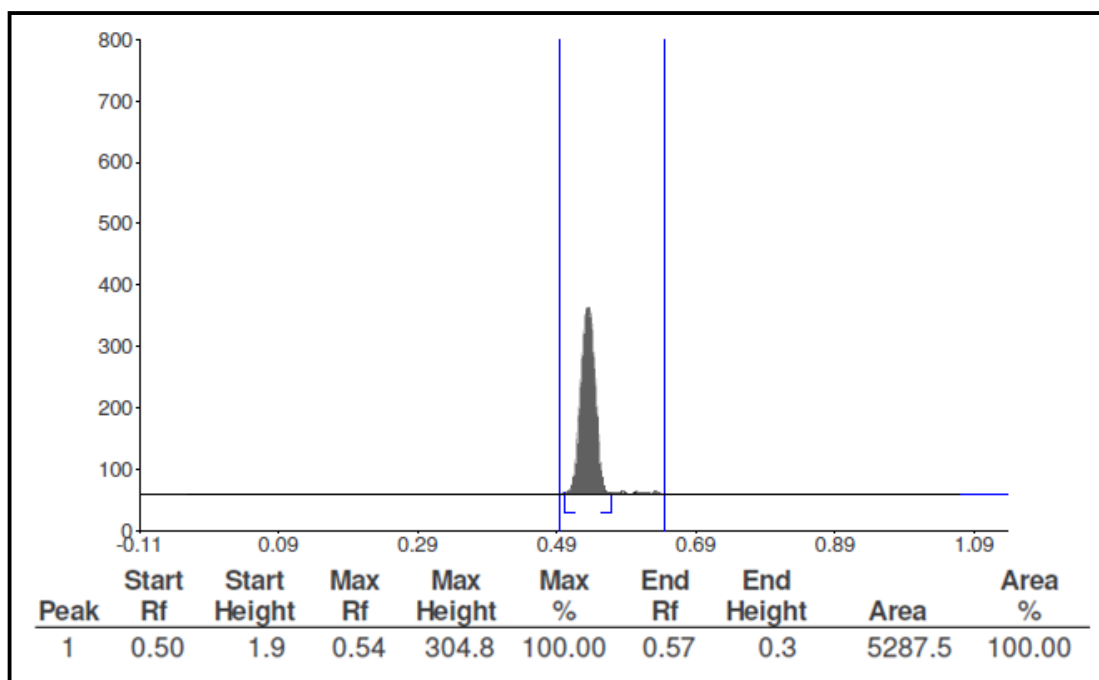**Figure No.4: Peak Area for Rosiglitazone**

CONCLUSION

The proposed HPTLC method is found to be simple, accurate, precise, linear, and specific, and, for quantitative estimation of rosiglitazone in bulk and its formulation.

ACKNOWLEDGEMENT

The authors thank Micro labs Pvt. Ltd, Badi, India, for providing a sample of rosiglitazone as a gift.

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