A Novel Validated RP-HPLC Method for Development and Estimation of Rosiglitazone in Pharmaceutical Dosage Forms and Rat Plasma

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Abstract **- A Simple and rapid RP-HPLC method has been developed and validation of rosiglitazone (ROZ) bulk and in pharmaceutical dosage forms. Metformin (MF) used as an internal standard (IS), the study was performed on a Develosil ODS HG-5 RP C18 (5□m,15cmx4.6mm i.d.) with a mobile phase of methanol: potassium dihydrogen phosphate (70:30v/v) as a mobile phase and was delivered at a flow rate of 1.0 ml/min. The detection was carried out at a wavelength of 245nm (Shimadzu-LC 10AD UV detector). The retention time of rosiglitazone was found to be 3.85min respectively. For rosiglitazone, the calibration curve was linear over the concentration range of 120-400 ng/ml for rosiglitazone (UV detector). The method was successfully developed and validated for rosiglitazone estimation, with improved sensitivity, linearity, accuracy and precision as per ICH guidelines and found to be convenient and effective for the estimation of rosiglitazone in pharmaceutical dosage forms and rat plasma.**

Index Terms **- Rosiglitazone (ROZ), Metformin (MF), Rat plasma and RP-HPLC.**

INTRODUCTION

Liquid chromatography is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the transit time of the solutes through a column [1].

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, and sensitivity, ruggedness [2, 3].

Rosiglitazone is an antidiabetic drug, belongs to class of thiazolidinedione. It works as an insulin sensitizer, by binding to the peroxisome proliferator activated receptors (PPARs) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver [4]. It is chemically designated as 5-[(4-{2-[methyl (pyridin-2-yl) amino] ethoxy} phenyl) methyl]-1, 3-thiazolidine-2, 4-dione. The chemical formula C18H19N3O3S and molecular weight is 357.427 gm/mol. Rosiglitazone soluble in dimethyl formamide, dimethyl sulfoxide, ethanol and pH 7.4 phosphate saline buffer [5].

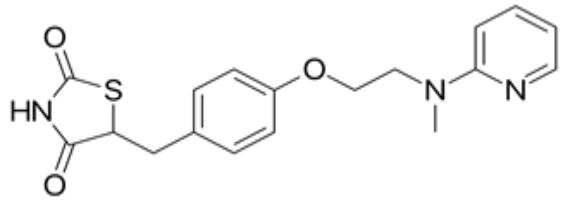

Figure 1: Structure of Rosiglitazone

Extensive literature survey proved that very few methods were reported for the estimation of rosiglitazone individually by RP-HPLC method. So we attempted to develop an accurate, rapid, precise, stable, sensitive and economically viable liquid chromatographic method for the estimation of rosiglitazone in pharmaceutical dosage form and in rat plasma in the present research.

MATERIALS AND METHODS

Materials:

Pharmaceutical grade pure rosiglitazone gift sample was procured from Taj pharmaceuticals Ltd, Mumbai,

India. Marketed formulation Tablets with dose of 8 mg of rosiglitazone was procured from local market (Avandia by Glaxosmithkline Inc). HPLC grade methanol was procured from Merck specialties private limited, Mumbai. Potassium dihydrogen orthophosphate was procured from Sd fine-Chem ltd; Mumbai.

Methods:
HPLC Method development:
Mobile Phase Optimization:
Initially the mobile phase tried was phosphate buffer and methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized with methanol and Phosphate buffer (pH 6.2), in proportion 70:30v/v respectively.

Selection of wavelength:
The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Rosiglitazone, so that the same wave number can be utilized in HPLC UV detector for estimating the Rosiglitazone [6]. While scanning the Rosiglitazone solution we observed the maxima at 245nm. The UV spectrum has been recorded on ELICO SL-159 make UV–Vis spectrophotometer model UV-2450.The scanned UV spectrum is attached below figure (Fig No 1).

Optimization of Column:
The method was performed with various columns like Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. was found to be ideal as it gave a good peak shape and resolution at 1.0 ml/min flow [7].

Optimized chromatographic conditions:
Instrument used: Waters HPLC with auto sampler and PDA Detector.
Temperature: Ambient
Column: Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Buffer: 136.09 gms of Potassium dihydrogen orthophosphate was dissolved in 1000 ml water pH adjusted with NaOH.
pH: 6.2
Mobile phase: 70% methanol 30% buffer
Flow rate: 1.0 ml per min
Wavelength: 245 nm
Injection volume : 20 µl
Run time : 6 min.

Preparation of buffer and mobile phase:
Preparation of Phosphate buffer:
Accurately weighed 136.09 gms of potassium dihydrogen ortho phosphate was taken into a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 6.2 with NaOH [8].

Preparation of mobile phase:
Accurately measured 300 ml (30%) of above buffer and 700 ml of methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 5 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as Diluent [9].
Preparation of Rosiglitazone standard and sample solution

Standard Solution Preparation:
25mg of rosiglitazone working standard was accurately weighed and transferred into a 25mL volumetric flask and about 20 ml of diluent was added to it and sonicated to dissolve drug completely and volume was made up to the mark with the same solvent which gave stock solution of 1000ppm. 1ml of the above stock solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents to prepare 100ppm solution. Further 1 ml of prepared 100ppm solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents which gave 10ppm rosiglitazone working standard solution. The solution was mixed well and filtered through 0.45µm filter [10].

Sample Solution Preparation:
Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25ml with same solvent. Then

10 ml of the above solution was diluted 11 to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five into the HPLC system [11].

Assay procedure:

A solution of 20 µL standard, sample separately were injected into the chromatographic system and areas for rosiglitazone peak was measured and the percentage assay calculated by using the formulae. Recorded the chromatogram and measured the peak responses [12]. Calculated the mean and percentage RSD for the same. These results are shown in below table

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where,AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of drug mg/ml.

Validation parameters:

Linearity

A standard curve of rosiglitazone was prepared, the slope, intercept, and the correlation coefficients were determined. The data were subjected to statistical analysis using linear regression model. For calculation of the standard curve a plot of peak area against concentrations were used. The calibration curves were obtained using Microsoft Excel 2007 software [13].

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification were calculated for rosiglitazone according to ICH guidelines by taking standard deviation and slope from the calibration curve. LOD and LOQ were calculated by using below formula, LOD-3.3×SD/slope; LOQ-10×SD/slope

Precision

Precision of the analytical method were determined by replicate processing. 3 different concentrations of rosiglitazone in triplicates were prepared and applied on pre coated silica gel plates. Precision was calculated as percent relative standard deviation. The relative standard deviation should be less than 2 according to ICH guidelines [2, 14].

Accuracy

Accuracy (recovery): To the pre analyzed sample add standard solutions in different levels (50%, 100% and 150%). These solutions were applied on precoated silica gel plate. The recovery of rosiglitazone was determined at 3 concentration levels (low, medium and high).

Robustness

The saturation time of mobile phase was increased to 1hr and the Rf should not change even if we increase the saturation time [15].

Rat serum studies of Rosiglitazone

Animals

Male Albino rats (250 -300 g) were purchased from Sainath agencies, Hyderabad, Telangana. The animals were maintained on a 12 hr light–dark cycle (light on from 8:00 to 20:00 h) at ambient temperature of 25 ± 2 °C and 50 ± 15% relative humidity. Rats were fed with a commercial pellet diet and water *ad libitum*. They were fasted overnight prior to the experiment and during the experiment, the food is withdrawn but not the water. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee [16].

Extraction procedures

Albino rats were administered with rosiglitazone as mentioned above. Blood samples (0.3 ml) were collected through retro-orbital plexus under mild ether anesthesia at a time period of 0, 1, 2, 3, 4, 8, 12 and 24 hr following drug administration using Sodium citrate (3.8%) as an anticoagulant. Plasma was separated immediately by centrifugation at 5000 rpm for 15 min and stored at -20° C until analysis. At the time of analysis, the stored plasma was used for extraction as described above [17].

To 100 µL of plasma samples, 20 µL of internal standard from 100 µg/ml of working solution was added and 400 µL of methanol was added, the resultant solution was mixed for 2 minutes on cyclomixer at room temperature and centrifuged at 5000 rpm for 15 min and the supernatant was separated and the supernatant is evaporated to dryness on water bath, the residue was dissolved in 100 µL of mobile phase and after filtration through 0.2 µm syringe filter, 20 µL of the solution was used for the HPLC analysis [18, 19, 20].

Grouping of Animals:
Albino rats were grouped as follows:
Group-I : Control (5% Gum Acacia 10ml/kg).
Group- II : Rosiglitazone (10mg/kg for single day).

RESULTS AND DISCUSSIONS

Method development for Rosiglitazone
The absorbance of rosiglitazone was scanned from wavelength of 200-400 nm using a Schimadzu UV-Vis spectrophotometer (UV ELICO SL210) and maximum absorbance was found at wavelength of 245 nm in methanol. Therefore, wavelength of 245 nm was chosen for the present study (Fig No 1). The mobile phase used for the study was of very simple composition and achieved optimal separation of rosiglitazone and internal standard (Metformin) without interference from the other components in plasma samples. The flow rate was selected as 1ml/min.

HPLC method validation:
Specificity and Sensitivity:
No interference of endogenous compound peaks was detected with rosiglitazone and I.S (Metformin) at their respective retention times (Rosiglitazone R_t = 3.855 min and I.S (Metformin R_t = 2.669 min) in blank rat plasma (Fig No 2, 3 & 4). From the chromatogram it was observed that the Metformin and Rosiglitazone peaks are well separated.

LOD and LOQ for rosiglitazone
The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio (LOD). The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio (LOQ). LOD was found to be 3.04 & LOQ was found to be 9.95 for rosiglitazone. Results were shown in below Table No.1 & Fig No 5 & 6.

Linearity of Rosiglitazone
The linearity data of rosiglitazone was given in (Table No 2). Calibration curve of rosiglitazone was given in (Fig No 7).The method showed linearity in the range of 120-400ng/µl for rosiglitazone and the correlation coefficient was found to be 0.999.

Precision of Rosiglitazone
Precision data of rosiglitazone given in (Table No 3).The %RSD of concentrations 1µg - 10µg/ml of rosiglitazone was less than 2. %RSD should be less than 2 according to ICH guidelines. So the method was found to be precise.
In order to assess the intra- and inter-day precision, rosiglitazone samples at low (1 µg/ml), medium (4 µg/ml) and high (10 µg/ml) concentrations were prepared as described above. The intra-day precision of the assay was assessed by calculating the coefficient of variation (CV) for the analysis of samples in three replicates and twice in a day. And inter-day precision was determined by the analysis of samples on three consecutive days. The results are found in table no 4.

Accuracy
Accuracy was calculated by comparing the measured values and the true values and was expressed in percent. Accuracy was accepted when the average values are > 95% of true concentration except for the LOQ where the limit was > 92%. The percentage Recovery for each level should be between 98.0 to 102.0%. Results were shown in table no.5.

Robustness
Robustness study was carried out by changing the wavelength (240 & 250 nm) and flow rate (1.2 and 1.5mL/min) of the mobile phase. Results were shown in Table No 6 & 7. The results were found to be satisfactory.

System Suitability Parameters for rosiglitazone
System suitability tests are an integral part of liquid chromatographic methods. These tests are used to verify that the chromatographic system is adequate for the intended analysis. Results were shown in Table No 8. The tests are based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such. The factors that may affect chromatographic behavior include the following are composition, ionic strength, temperature, apparent pH of the mobile phase, flow rate, column dimensions, column temperature and pressure.

Assay of rosiglitazone in dosage form
The assay of Avandia tablets containing rosiglitazone was found to be 100.065 %. Results obtained are tabulated in Table No 9.

Rat serum studies:

The concentration of rosiglitazone in extracted plasma was determined and the graph was plotted by taking Time in hrs on X- axis and corresponding average concentration (µg/ml) values of drug in plasma samples on Y-axis. Our study showed that the rosiglitazone showed has a significant effect in treated rats and compared with control group. The results are shown in Fig No 8 and data represented in Table No 10 & 11.

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Rosiglitazone API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of rosiglitazone in different formulations. The results obtained on the validation parameters met ICH requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision. From the present study we can conclude that there is significant effect of rosiglitazone was observed in rat plasma studies for 24 hrs time period and compared with control.

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Titles of Figures:

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8) Figure No 8: Graph showing Time Vs Concentration profile of Rosiglitazone and Control.

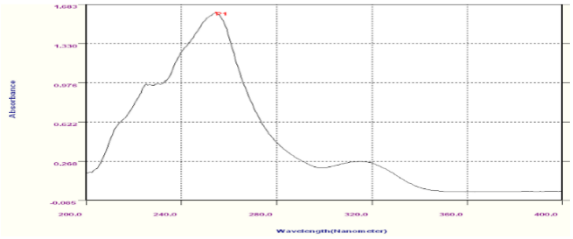

Figure No 1: UV spectrum for Rosiglitazone

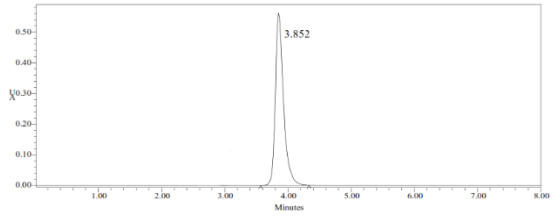

Figure No 2: Chromatogram for standard of Rosiglitazone (RT 3.852)

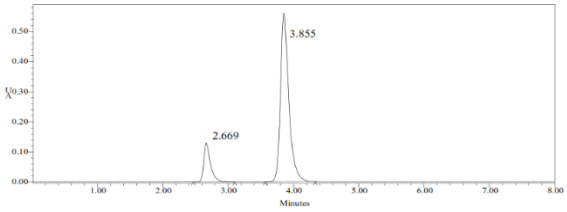

Figure No 3: Chromatogram for Metformin (IS) and Rosiglitazone (API)

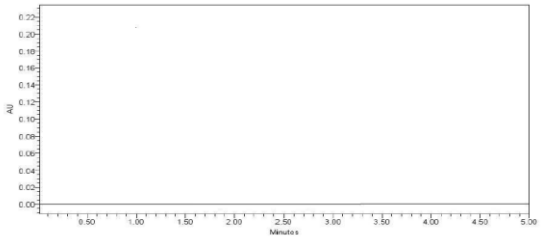

Figure N0 4: Chromatogram for blank

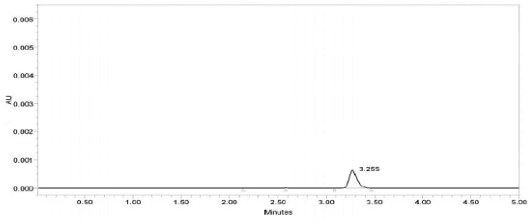

Figure No 5: Chromatogram of Rosiglitazone showing LOD

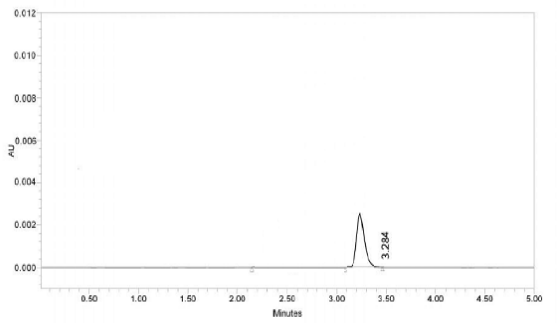

Figure No 6: Chromatogram of Rosiglitazone showing LOQ

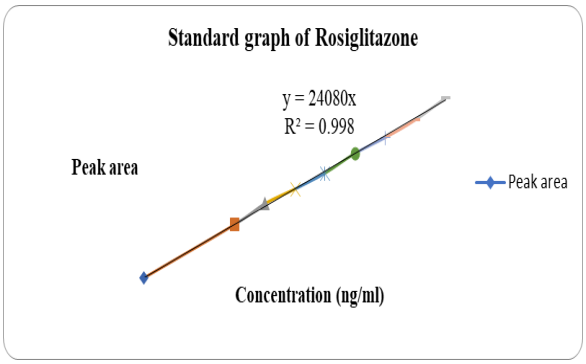

Figure No 7: Construction of standard graph of Rosiglitazone

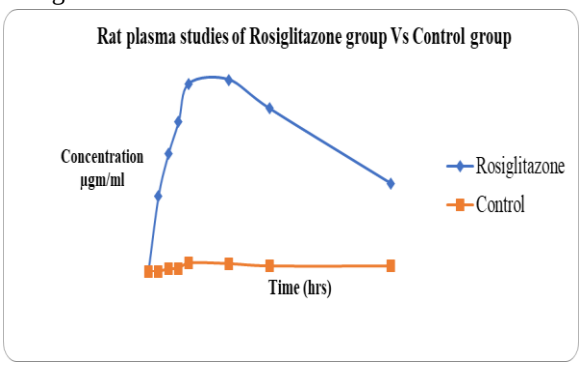

Figure No 8: Graph showing Time Vs Concentration profile of Rosiglitazone and Control

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Table No 1: LOD & LOQ for Rosiglitazone

Drug name		Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Rosiglitazone	LOD	46 µV	140 µV	3.04
	LOQ	46 µV	458 µV	9.95

Table No 2: Linearity data of Rosiglitazone

Conc.(ng/ml)	Peak area
0	0
120	3007510
160	4080477
200	4852277
240	5667612
280	6340819
320	7614892
360	8372639
400	9756921

Table No 3: Precision Study of Rosiglitazone

Replicates of Rosiglitazone	Retention Time	Area
Replicate – 1	3.85	4852277
Replicate – 2	3.86	5110171
Replicate – 3	3.84	4890990
Replicate – 4	3.83	5028389
Replicate – 5	3.87	4987596
Average	3.85	4973885
Standard Deviation	0.008944	93158.62
% RSD	0.254532	1.872955

Table No 4: Results of intra-day & inter-day precision

Conc. of Rosiglitazone (API) (µg/ml)	Observed conc. of Rosiglitazone (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
1	10.005	1.05	10.006	0.24
4	30.003	0.55	30.084	0.41
10	99.84	0.18	99.95	0.18

Table No 5: Accuracy data for Rosiglitazone

% of concentrationLevel (n=3)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	2824068	8.0	8.07	101.36 %	99.88 %
100%	5565330	10.0	9.99	99.87 %	
120%	7956251	12.0	12.56	100.8 %	

Table No 6: Variation in wavelength results for Robustness

Wavelength	Drug	Theoretical Concentration µg/ml	Mean (Peak area)	Rt value	%RSD
240 nm	Rosiglitazone	10	1973251	5.51	0.79
		30	5287114	5.49	0.68
		10	1973197	5.48	1.04
250 nm		30	5286939	5.54	0.88

Table No 7: Variation in flow results for Rosiglitazone for Robustness

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.0	2461	1.55
2	1.2	2531	1.67
3	1.5	2406	1.45

Table No 8: Results of system suitability parameters for Rosiglitazone

S.No	Name	Retention time (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Rosiglitazone	3.85	1308495	154566	6 0	1.3	6090.3

Table No 9: Assay of Rosiglitazone tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Avandia	8	200.13 (±0.06)	100.065 (±0.48)

Table No 10: Concentration and time profile of rosiglitazone after oral administration (24hrs time period)

Time (hr)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean	SD
0	0.0	0.0	0.0	0.0	0.0	0	0.0
1	25.17	28.35	20.92	35.15	35.59	29.036	3.206213
2	45.72	48.12	45.06	42.15	46.27	45.464	4.235062
3	50.73	51.92	63.52	57.64	65.53	57.868	5.837471
4	75.98	78.36	69.24	70.78	68.64	72.600	3.971824
8	68.51	65.96	81.53	75.97	78.45	74.084	5.676865
12	56.94	62.58	59.23	70.16	65.72	62.926	4.281976
24	35.12	35.48	32.65	35.96	31.25	34.092	2.149531

Table No 11: Concentration and time profile of control after oral administration (24 hrs)

Time (hr)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean	SD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.08	0.05	0.17	0.19	0.10	0.118	0.58
2	1.82	1.65	0.39	0.72	0.69	1.054	0.45
3	1.43	1.51	0.71	0.91	1.36	1.184	0.82
4	2.94	3.78	2.95	3.98	2.65	3.260	0.72
8	2.82	2.65	3.82	2.91	2.62	2.964	0.48
12	1.75	1.94	2.62	2.29	1.85	2.090	0.52
24	2.52	2.64	1.73	1.51	1.82	2.044	0.28