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RP-HPLC METHOD DEVELOPMENT AND ESTIMATION OF ROSIGLITAZONE MALEATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple and accurate RP-HPLC method has been developed for the estimation of Rosiglitazone Maleate (RGZ) in tablet pharmaceutical dosage form using 100; C_{18} (250 x 4 mm, 5µm) column with mobile phase comprising of acetonitrile: 0.01M ammonium acetate in the ratio 50:50 v/v. The flow rate was 1.0 ml/min and detection was carried out by UV-PDA detector at 245nm. The retention time for RGZ was found to be 3.008min. The linearity range, correlation co-efficient and accuracy of RGZ was found to be 01-200 µg/ml, 0.9992 and 99.75 – 105.3% respectively. The developed method was found to be simple, precise and accurate for the estimation of RGZ in tablet formulations.

Keywords: Rosiglitazone Maleate, RP-HPLC, tablet pharmaceutical dosage form, method development, validation

1. INTRODUCTION

Rosiglitazone Maleate (RGZ) is an antidiabetic drug in the thiazolidinedion class for oral administration **[1]**. RGZ is chemically (±)-5-{*p*-[2-(Methyl-2-pyridylamino) ethoxy]benzyl}-2,4-thiazolidinedione maleate. Structure shown in (**Figure 1**).

RGZ acts as a highly selective and potent agonist at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action on skeletal muscle, adipose tissue and liver. PPAR-gamma receptor activation controls the transcription of insulin-

responsive genes involved in the control of glucose production, utilization and transport. In this way, RGZ enhances tissue sensitivity to insulin. RGZ is used in the treatment of diabetes Type 2, diabetes mellitus, diabetic coma and acute diabetic ketoacidosis. Molecular formula and the molecular weight of RGZ are $C_{18}H_{19}N_3O_3S$ and 473.5gm/mol respectively **[2]**. Literature review reveals that very few analytical methods were evoked for the determination of RGZ by RP-HPLC method for the determination of RGZ in pharmaceutical dosage forms, UV-Visible Spectrophotometric determination of RGZ in pharmaceutical formulations **[3]**. The present study was aimed to develop a simple and reliable RP-HPLC for the determination of RGZ in their tablet dosage forms.

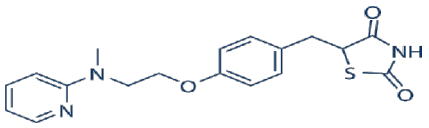

Figure 1: Structure of RGZ

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

RGZ (99.999% purity) was obtained as a gift sample from Arubindo laboratories, Pydibimabaram, Andhra pradesh. methanols, acetonitrile, 0.01M Ammonium acetate, were of HPLC grade obtained from Merck research laboratory, Mumbai, India. Water (HPLC Grade) obtained from Himedia Pvt. Ltd, India.

2.2 Instrumentation

Quantitative HPLC was performed on an isocratic mode HPLC with Shimadzu LC-10AT and LC-10AT VP series HPLC pumps, with a 20µl sample loop (manual), and SPD 10A VP UV-Visible absorbance detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. A LiChroCART® LiChrospher® 100; C18 (250 x 4 mm, 5µm) column was used for the separation.

2.3 Chromatographic Conditions

To develop a suitable RP-HPLC method for the determination of RTZ different mobile phases like methanol: water (50:50), methanol: 0.01M ammonium acetate (50:50), acetonitrile: water (50:50) and acetonitrile: 0.01M ammonium acetate (50:50) were tried at different flow rates (0.8,1.0 ml/min). The mobile phase in the composition of acetonitrile: 0.01M ammonium acetate in the ratio 50:50 at a flow rate of 1.0ml/min gave sharp peaks with minimum tailing compared to the other one. The retention time for RGZ was found to be 3.008min. The UV detection λmax for the drug was determined by scanning different concentrations solution of the drug in the mobile phase and was found to be 245nm. The optimized chromatographic conditions are given in **(Table 1)**.

Table 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	LiChroCART® LiChrospher® 100;C_{18} (250 x 4 mm,5µm)
Mobile Phase	Acetonitrile: 0.01M ammonium acetate(50:50)
Flow rate (ml/min)	1.0
Column back Pressure(kgf) (kgf/cm^2)	167
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	245
Drug RT (min)	3.008

2.4 Chromatographic Method

2.4.1 Preparation of Mobile Phase

0.3854gm of Ammonium acetate was taken in 500ml volumetric flask and properly mixed with 500ml triple distilled water and sonicated for 30minutes for degassing followed by filtration. Acetonitrile HPLC grade was properly ultrasonicated for 30mins degassing followed by filtration a 0.45µ membrane filter.

2.4.2 Preparation of Stock Solutions

25mg of RGZ pure drug was weighed and dissolved in 15ml of mobile phase and made upto the mark in 25ml of volumetric flask to get a concentration of 1000µg/ml.

2.4.3 Preparation of Working Standard Solutions and Procedure for construction of Calibration Curve

The solution was further diluted with the mobile phase in order to achieve a working standard solution of RGZ. The dilutions were prepared in the concentration range of 1µg to 200µg/ml. The contents of the mobile phase were filtered through Whatman filter paper (0.45µm) consisting of cellulose nitrate and pumped at a specified flow rate from the respective solvent reservoirs to the column. The column was equilibrated for at least 30 minutes with the mobile phase running through the systems before the drug solutions were injected. A mobile phase made up of acetonitrile: 0.01 M ammonium acetate (50:50) v/v with a flow rate of 1 ml/min was used for chromatographic separation. Using UV detector, the eluent was monitored at a 245 nm wavelength. The column was maintained an ambient temperature (250C) and an injection volume of 20 ml each of standard and sample solution was injected into the HPLC device to obtain chromatograms. During the retention time, average peak areas of drug were recorded. The graph was plotted by taking the concentration of the drug in the X-axis and the peak area in the Y-axis. For RGZ, the linearity range was found to be between 1-

200(g / ml). The linearity range and linearity graph were shown in **Table 2 & Figure 1** respectively. A typical chromatogram of RGZ (10µg/ml) (Pure drug) is shown in **(Figure 2)**.

2.4.4 Preparation of Test solution

For analysis of commercial formulation, 20 tablets containing RGZ of marketed formulation were taken and powdered. The powder equivalent to 10mg of the active ingredient was correctly weighed and taken in to a 100ml volumetric flask and mobile phase was added. for complete dissolution of the drug, volumetric flask was sonicated for 30 minutes. Finally the solution was made up to the mark with mobile phase and then filtered through Whatman filter paper (0.45µm) made up of cellulose nitrate. Appropriate aliquots were then transferred to a 10ml volumetric flask and made up to volume with mobile phase to yield concentrations of drug in range of linearity previously described. The amount of drug present in each pharmaceutical formulation was calculated by using the standard calibration curves (concentration in µg/ml was taken on X-axis and peak area on Y-axis). A typical chromatogram of RGZ (10µg/ml) (Formulation) is shown in (**Figure 3)**.

2.4.5 Linearity

The linear fit of the system has been graphically demonstrated. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. The results are presented in (**Table 2)**.

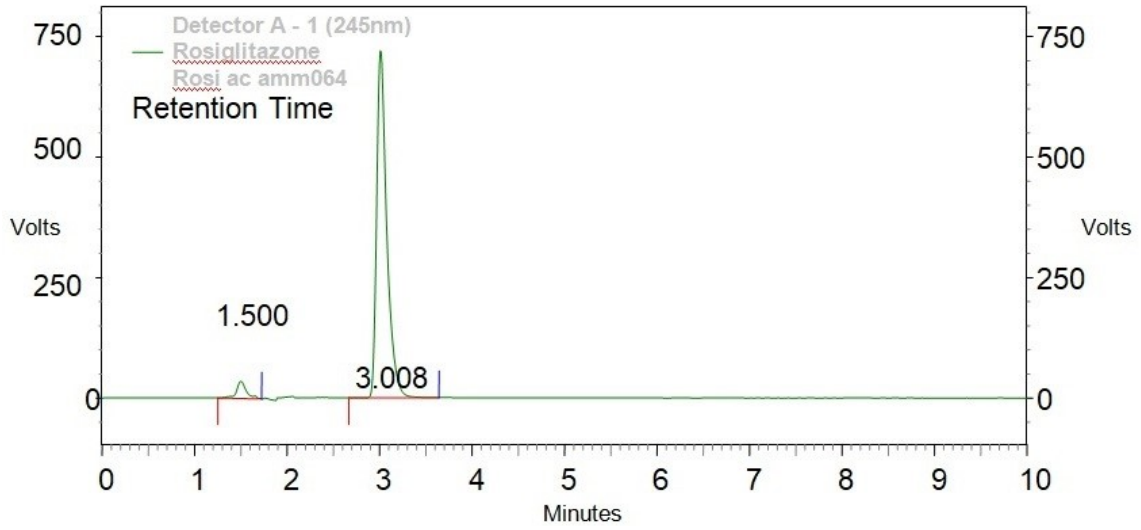

Figure 2: Representative Chromatogram of RGZ (10µg/ml) (Pure Drug)

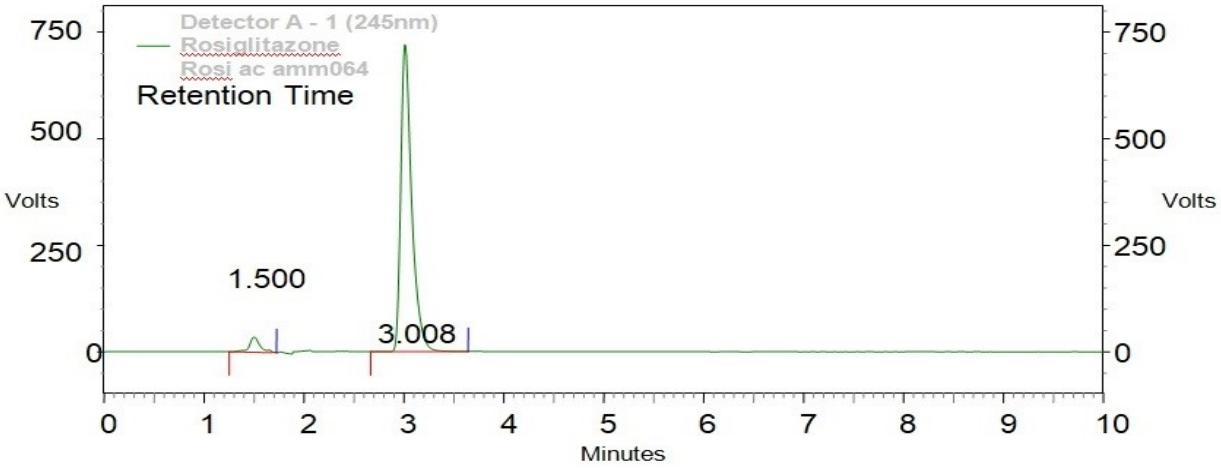

Figure 3: Representative Chromatogram of RGZ (10µg/ml) (Pure Drug)

3. RESULTS AND DISCUSSION

3.1 Method Development

The above RP-HPLC procedure was developed for the determination of concentration of RGZ in tablet dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios of solvent were used to get optimized mobile phase. Finally a mixture of the mobile phase in the composition of acetonitrile: 0.01M ammonium acetate in the ratio 50:50 v/v at a flow rate of 1.0ml/min was optimized. A typical chromatogram was obtained by using the above mentioned mobile phase which was illustrated in (**Figure 2**) and the retention time was found to be 3.008 minutes.

3.2 Method Validation

After the development of RP-HPLC method, it has been validated in terms of parameters like specificity, precision, accuracy, linearity, range, ruggedness, robustness and stability. For all the parameters, percentage relative standard deviation values were calculated. The proposed RP-HPLC method was validated as per ICH guidelines.

3.2.1 Linearity and Range

The linearity was checked by evaluating various concentrations of the standard solutions of RGZ. The Beer's Lambert's concentration was found to be 01 to 200 µg/ml. Calibration curve was drawn by plotting average peak area against concentration and regression equation was calculated **[4]**. The graph was given in **(Figure 4)** and linearity in **(Table 2)**.

From the graph **(Figure 4)**, it was noted that an excellent correlation exists between peak area and concentration of drug. Slope and the R^2 value of RGZ were found to be 0.9992. It

was observed that the regression value obtained was found to be within the limit.

3.2.2 Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly with multiple samplings of a homogenous sample. Precision of RGZ was evaluated and the percentage relative standard deviation (% RSD) was found to be less than 1% which proves that the method was precise **[5]** (**Table 3**).

3.2.3 Accuracy

In order to determine the accuracy of the proposed procedure, recovery studies were performed by taking different amounts (80%, 100%, and 120%) of bulk samples of RGZ within the linearity range and adding to the pre-analyzed formulation concentration. From that percentage recovery values were calculated **[6]**. The results are shown in **(Table 4)**.

3.2.4 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The retention times of various excipients added to the tablet formulation of RGZ does not interfere with the retention time of the active ingredient **[7]**. Specificity of the RGZ was shown in (**Figure 5**).

3.2.5 System suitability

System suitability is the test to ensure that the method can generate results of precision and acceptable accuracy. The System performance parameters of the developed RP-HPLC method have been determined by analyzing standard working solutions. System suitability can be measured by determining the chromatographic parameters such as tailing factor ,plate number (N), resolution (Rs) and relative standard deviation of peak height or peak area for repetitive injections System suitability of RGZ is given in (**Table 5**).

3.2.6 Analysis of a marketed preparation

The results obtained for the amount of RGZ in tablet powder against the label claims were in good agreement it indicates that there is no interference from the excipients presents in the tablet **[8]**. For RGZ the percent assay was found to be 99.25% (**Table 6**).

Table 2: Linearity Table for RGZ by RP-HPLC method

Concentration (µg/ml)	Peak Area (Drug)	Statistical Analysis
0	0	Slope (a) :83973 Intercept (b):63823 Correlation coefficient : 0.9992
1	89600	
5	453003	
10	897271	
20	1739878	
40	3309084	
60	5063626	
80	7018168	
100	8672710	
150	12859065	
200	16545420	

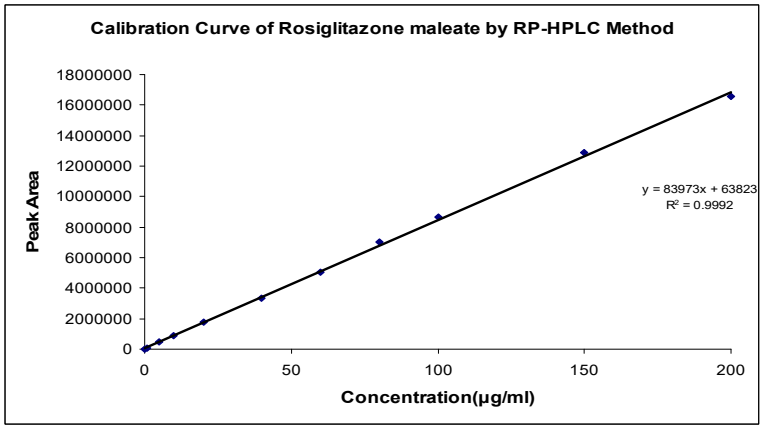

Figure 4: Calibration curve of RGZ

Table 3: Percentage relative standard deviation of RGZ

S. No.	Concentration (µg/ml)	Peak Area (Drug)	Statistical analysis
1	10	897643	Mean =897742 S.D. = 18.824 % R.S.D. = 0.00209
2.	10	897836	
3.	10	897789	
4.	10	897698	
5.	10	897762	
6.	10	897801	
7.	10	897732	
8.	10	897781	

Table 4: Accuracy of RGZ

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	105	Mean= 104.86% S.D. = 0.5131 % R.S.D.= 0.4893
S_2 : 80 %	8	10	105.3	
S_3 : 80 %	8	10	104.3	
S_4 : 100 %	10	10	99.9	Mean= 100.06% S.D. = 0.2081 % R.S.D.=0.2079
S_5 : 100 %	10	10	100	
S_6 : 100 %	10	10	100.3	
S_7 : 120 %	12	10	100	Mean= 100.44% S.D. = 0.9922 % R.S.D. = 0.9878
S_8 : 120 %	12	10	99.75	
S_9 : 120 %	12	10	101.58	

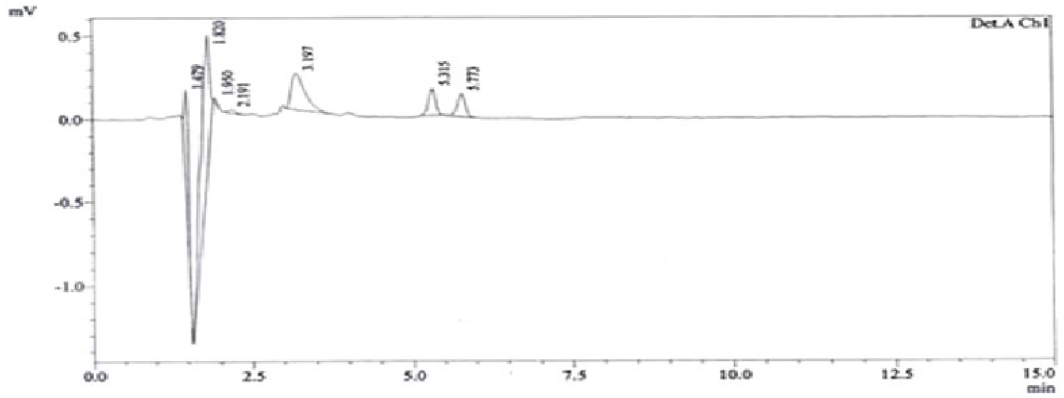

Figure 5: Chromatogram for Specificity obtained by injecting the Placebo

Table 5: System suitability of RGZ

Parameters	Obtained Values
Theoretical plates (N)	2194
Tailing factor (T)	1.1
LOD (µg/ml)	0.022
LOQ (µg/ml)	0.076

Table 6: Analysis of Commercial Formulation

Formulation	Labeled Amount (mg)	Observed Amount* mg ±S.D.	% Recovery by proposed method	% RSD
ROGLIN Tablet (Aristo)	4	3.97 ± 0.0028	99.25	0.0705

4. CONCLUSION

For the determination of RGZ from pure and its dosage forms, the proposed method was found to be simple, rapid, accurate and precise. The mobile phase is simple, convenient to prepare and economical. The recovery of the sample in all formulations was in good agreement with their respective label assert and specify that the formulation should not be interfered with in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of RGZ in pure form and its dosage forms and can also be used for dissolution or similar studies.

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Conflict of interest

The authors declare that they have no conflict of interest. The article does not contain any studies with animals or human participants performed by any of the authors.

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