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# Development and validation of new RP-HPLC method for determination of rosiglitazone HCl in pharmaceutical dosage forms

**V Jagathi[1]*, P Sai Praveen[1], V Rajesh[2], D Ramesh[1], Jahnavi G[1]**

[1]K.V.S.R.Siddhartha College of Pharmaceutical Sciences, Vijayawada – 520 010, A.P, India.
[2]MIC College of Technology, Kanchikacherla,Vijayawada – 521180, A.P,India.

**ABSTRACT**

A simple and precise RP-HPLC method was developed and validated for the determination of Rosiglitazone hydrochloride in pharmaceutical dosage forms. Chromatography was carried out using Princeton SPHER C18 (250 x 4.6 mm i.d., 5µ), buffer: acetonitrile (73:27) as the mobile phase at a flow rate 1.0 ml/min. The analyte was monitored using UV detector at 243 nm .The Retention time of the drug was 6.4min for Rosiglitazone hydrochloride. The proposed method was found to have linearity in the concentration range of 10– 60 µg/ml with correlation coefficient of r2=0.9998. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Rosiglitazone hydrochloride were in the range 100.09-103.11%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Rosiglitazone hydrochloride in bulk and dosage forms.

**Keywords:** Rosiglitazone hydrochloride, RP-HPLC.

***Corresponding author***
Email: vallurijagathi@gmail.com

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## INTRODUCTION

Rosiglitazone, 5-[[4-[2-(methyl-pyridin-2-ylamino) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4-dione is official in Indian Pharmacopoeia. Rosiglitazone acts as an agonist at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPAR-gamma receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In this way, rosiglitazone enhances tissue sensitivity to insulin. Rosiglitazone has a melting point of 122-123$^{o}$C. The molecular formula is $C_{18}H_{19}N_3O_3S$ and the molecular weight is 357.42. It was observed that Rosiglitazone is soluble in methanol and acetonitrile.

Only very few HPLC methods have been reported in the literature for the estimation of RGL present in biological fluids. There are no reported methods for the determination of RGL by HPLC in pharmaceutical dosage forms. Hence the author has made an attempt to develop a HPLC method for the determination of RGL in pharmaceutical formulations.

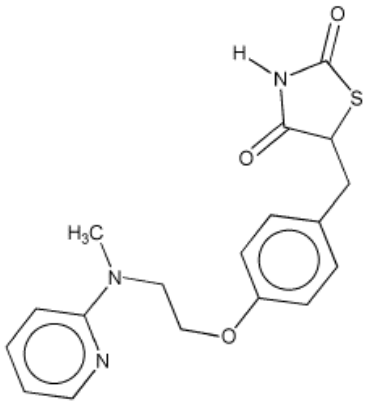


**Figure 1**

## MATERIALS AND METHODS

### Instrumentation

Alliance-Waters 2695 separation module with Waters 2996 U.V-Vissible detector equipped with EMPOWER software.

### Chemicals and reagents

Rosiglitazone hydrochloride was obtained as a gift sample from PharmaZell R&D centre, India Pvt.Ltd. water (HPLCgrade), acetonitrile (HPLC grade) and sodium acetate (AR grade).

**Chromatographic conditions**

Mobile phase consists of Sodium Acetate buffer, acetonitrile in the ratio (73:27). Buffer was prepared by dissolving 3.0 g of sodium acetate and dissolve it in 500ml of HPLC grade water. Adjust the pH to 5 ,filter through 0.45µm nylon membrane filter and degas.

The mobile phase was pumped from the solvent reservoir to the column at a flow rate 1.0 ml/min. The column was maintained at 45°C and the volume of each injection was 20µL. Prior to injection of the solutions, column was equilibrated for at least 20min with mobile phase flowing through the system. The eluents were monitored at 243nm.

Diluents: Mobile phase

**Standard Preparation**

Stock solution of Rosiglitazone HCL was prepared by dissolving about 10.0 mg of RGL working standard into a 100 ml clean dry volumetric flask, add about 60 ml of diluent, sonicate for 5 minutes, and dilute to volume with acetonitrile.

**Sample Preparation**

10 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder, dose equivalent to 100mg was transferred to a 50 ml volumetric flask, dissolved in working mobile phase and then the solution was made up to the mark with mobile phase and filtered through 0.45 µ membrane filter. 5 ml of this solution was pipetted into 20ml volumetric flask and diluted with the mobile phase to get concentration of 500 µg/ml [1-11].

## RESULTS AND DISCUSSION

Several systematic trials were performed to optimize the Chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Rosiglitazone HCL in pharmaceutical dosage forms. The present method contains mobile phase sodium acetate and acetonitrile in the ratio (73:27v/v) which was found to be the most suitable as the chromatographic peak obtained with good shape and symmetry. Hence this method was finalized for the estimation of RGL at retention time of 6.4 mins.

**Linearity**

The linearity graphs for the proposed assay methods were obtained over the concentration range of 10 – 60 µg/ml. The linearity graph was given in fig 3.2-3.7 Method of least squares analysis was carried out for getting the slope, intercept and correlation coefficient values and the results were presented.

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**Accuracy**

To determine the accuracy of the proposed method, different amounts of bulk samples of RGL n between the upper and lower linearity limits were taken and analyzed by the proposed method.

**Precision**

The precision of the method was ascertained from the peak area of RGL obtained by determination of six replicates of fixed amount of RGL. The percent relative standard deviation and percent range of errors (0.05 and 0.01 confidence limits) were calculated and were presented.

**Robustness**

Robustness of the proposed methods was evaluated by making small changes in flow rate, buffer concentration, pH of the buffer solution, organic modifier concentration and temperature. The results were found to be not affected by these small alterations.

## CONCLUSION

From the obtained results it can be concluded that the proposed method is quite precise and accurate. The absence of additional peaks in the Chromatogram indicated that there is no interference of the common excipients used in the tablets. The proposed HPLC method is sensitive and reproducible for the analysis of Rosiglitazone HCL in Tablet dosage forms. The method was duly validated by using required statistical parameters.

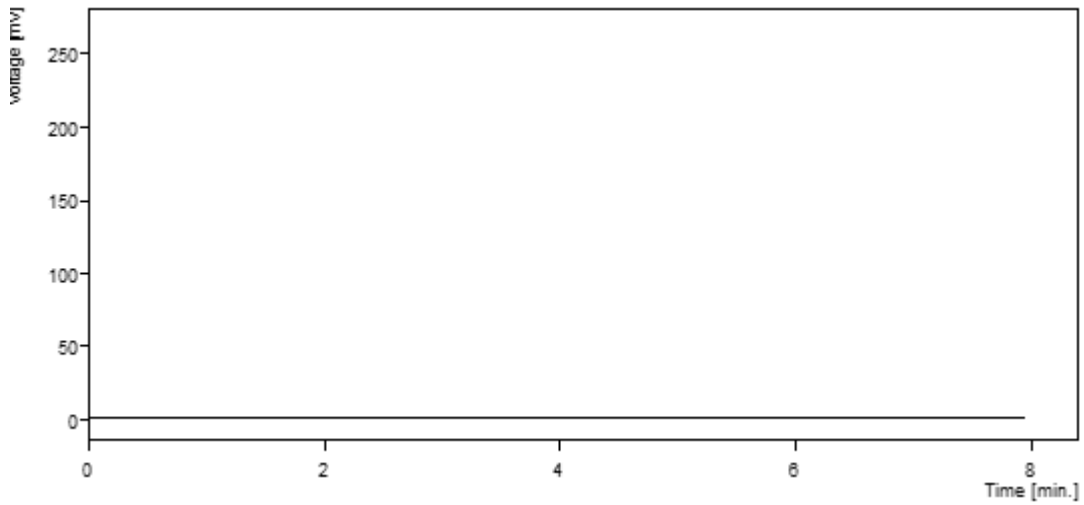


**Figure 2a Rosiglitazone blank**

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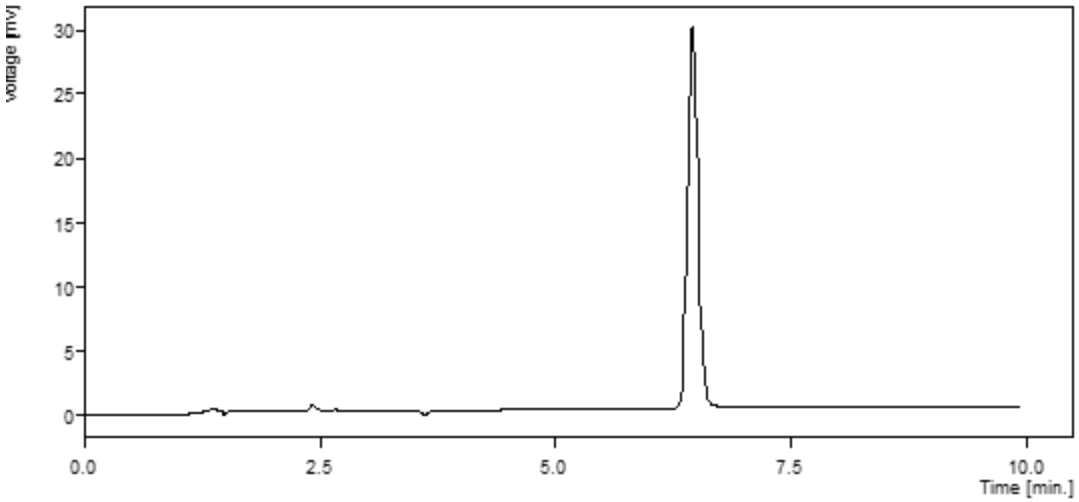


**Figure 2b Rosiglitazone standard**

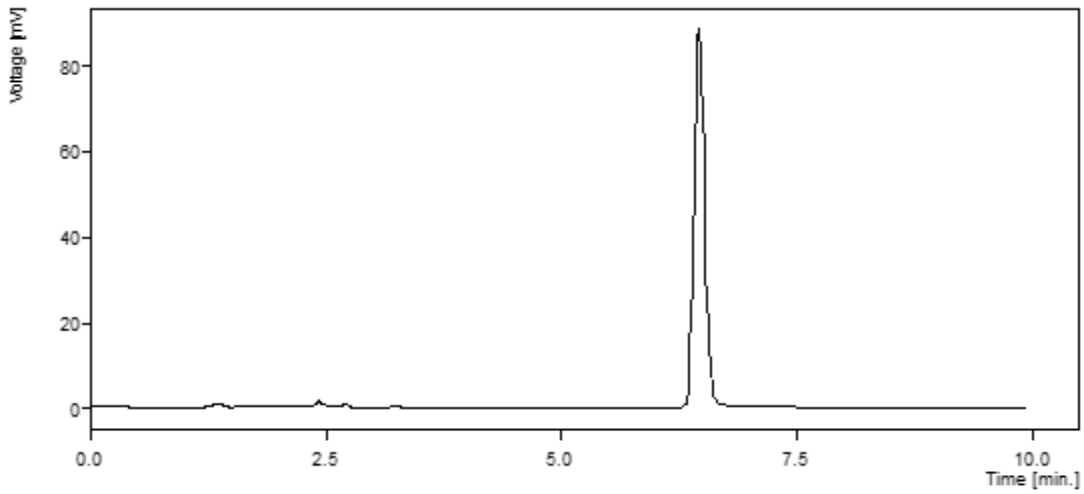


**Figure 2c Rosiglitazone sample**

**SYSTEM SUITABILITY AND SYSTEM PRECISION**

**System suitability, precision and accuracy of the proposed methods for** RGL

| Parameter | Results |
|---|---|
| Retention Time (t) (Min) | 6.48 |
| Theoretical Plates (n) | 13777 |
| Plates per Meter (N) | 52840 |
| Peak asymmetry | 1.182 |
| Resolution Factor | - |
| Linearity range (µg/ml) | 10– 50 |
| Detection limits (µg/ml) | 0.6356 |
| Regression equation (Y = a + bc) | |
| Slope (b) | 23.84 |
| Standard deviation of slope ($S_b$) | $1.517 \times 10^{-1}$ |
| Intercept (a) | 4.815 |

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| | |
|---|---|
| Standard deviation of intercept ($S_a$) | 4.594 |
| Standard error of estimation ($S_e$) | 6.3472 |
| Correlation Coefficient (r) | 0.9998 |
| (%) Relative standard deviation * | |
| Retention time | 0.06632 |
| Peak area | 0.0205 |
| Percentage range of errors* (Confidence limits) | |
| 0.05 level | 0.0214 |
| 0.01 level | 0.03367 |
| % Error in bulk samples** | 0.042 |

* Average of six determinations.

** Average of three determinations.

**TABLE 2.13**
**ASSAY RESULTS OF RGL IN PHARMACEUTICAL FORMULATIONS**

| Sample | Pharmaceutical Formulation | Labeled amount(mg) | Amount found* ± S.D. | Reference method | %Recovery ± R.S.D. |
|---|---|---|---|---|---|
| Brand I | Tablets I | 4 | 3.52 ± 0.025<br>t = 0.79, F = 2.16 | 3.85 ± 0.016 | 99.2 ± 0.229 |
| | Tablets II | 8 | 6.05 ± 0.036<br>t = 1.33, F = 2.66 | 6.05 ± 0.032 | 99.6 ± 0.356 |
| | Tablets I | 4 | 3.02 ± 0.017<br>t = 0.82, F = 2.07 | 3.01 ± 0.016 | 100.1 ± 0.27 |
| | Tablets II | 8 | 7.03 ± 0.031<br>t = 0.74, F = 1.52 | 7.89 ± 0.037 | 99.3 ± 0.312 |
| Brand II | Tablet I | 4 | 3.95 ± 0.018<br>t = 0.88, F = 1.98 | 3.87 ± 0.016 | 98.6 ± 0.236 |
| | Table II | 8 | 6.05 ± 0.036<br>t = 1.15, F = 2.23 | 6.69 ± 0.029 | 98.7 ± 0.254 |
| | Tablets I | 4 | 3.06 ± 0.041<br>t = 0.746, F = 2.56 | 3.01 ± 0.032 | 100.04 ± 0.119 |
| | Tablets II | 8 | 7.05 ± 0.036<br>t = 1.215, F = 2.88 | 6.86 ± 0.014 | 100.3 ± 0.216 |

* Average ± standard deviation of eight determinations, the t and F – values refer to comparison of the proposed method with Reference method. Theoretical values at 95 % confidence limits t = 2.365 and F=4.88.

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