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Rapid and sensitive RP-HPLC analytical method development and validation of Pioglitazone hydrochloride

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ABSTRACT

A simple, specific, accurate and isocratic reversed phase - HPLC method was developed and subsequently validated for the determination of Pioglitazone Hydrochloride. Separation was achieved with an Symmetry - Extend - C_{18} HPLC column 150mm in length and having an internal diameter of 4.6mm. A mobile phase comprising 0.01M Buffer: Methanol in the volume ratio of (40:60) was developed. The detection was carried out using a UV-detector set at a wavelength of 240nm. Validation experiments were performed to demonstrate System suitability, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 1-200µg/ml and get the correlation Regration (r^2) 0.999, showed good recoveries (99.3 - 103.2%). The method can be used for quality control assay of Pioglitazone Hydrochloride.

Key words: RP-HPLC, Pioglitazone Hydrochloride, Method Validation.

INTRODUCTION

Pioglitazone (PIO) is chemically (Fig. 1) (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2, 4-dione, and it is a prescription drug of the class Thiazolidinedione (TZD) with hypoglycemic (Antihyperglycemic, Antidiabetic) action. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α [1, 2].

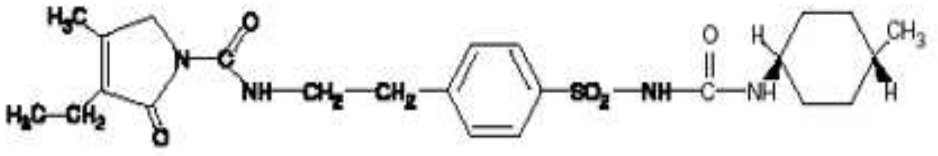

Fig. 1: Structure of Pioglitazone

It is official in United States Pharmacopeia [3]. And the pharmacopoeia describes HPLC method for estimation of PIO. A literature survey revealed human plasma [4-6] and HPLC method for Antidiabetic drugs [7-9] were reported.

Pharmaceutical validations among these methods undergo the world 'Validation' means 'Assessment' of validity or action of providing effectiveness [10, 11], and validation as per ICH guidelines [12].

MATERIALS AND METHOD

Apparatus:
The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 744 (metrohm), the HPLC used is of Younglin with UV-detector. Column used in HPLC is of 150mm × 4.6mm 5µ(Symmetry, ODS-3V, 150mm ×4.6mm, 5µ is suitable) with a flow rate of 1.2ml/min (Isocratic). The mobile phase consists of the mixture of Methanol and the Buffer pH (6.0) at different proportions are degassed in a sonicator for about 10minutes the injection volume is 20µl and the ultra violet detection was at 240nm.

Reagents and solutions:
Pure sample of Pioglitazone hydrochloride USP of 100mg and other ingredients such as Methanol and water used were of HPLC and milli-q grade. All other chemicals like Glacial acetic acid, Potassium Di-hydrogen phosphate used were of AR grade. Optimized chromatographic conditions are listed in table no.1.

Preparation of standard solution:
Accurately weighed 10mg of the Pioglitazone HCl reference standard was transferred to 10ml clean and dry volumetric flask. Then the volume was made up to the mark with the diluent and mixed well. This yielded standard stock solution with concentration 1000µg/ml of Pioglitazone HCl .From the stock solution 1ml was taken and it transferred to the 10ml clean and dry volumetric flask. Then the volume was made up to the mark with the diluent and mixed well. This yielded a standard solution with concentration 100µg/ml was injected.

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution and robustness.

Linearity & Range: The Linearity of detector response is established by plotting a graph to concentration versus area of Pioglitazone hydrochloride standard and determining the correlation coefficient. A series of solution of Pioglitazone hydrochloride standard solution in the concentration ranging from about 1-200µg/ml level of the target concentrations were prepared and injected into the HPLC system.

Accuracy: Accuracy for the assay of Pioglitazone hydrochloride tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Pioglitazone hydrochloride standard is added at different levels (80%, 100%, and 120%).

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

RESULTS AND DISCUSSION

Pioglitazone hydrochloride standard having concentration 100µg/ml was scanned in UV- region between 200-400nm. λ_{max} of Pioglitazone hydrochloride was found to be at 240nm. Pioglitazone hydrochloride Retention time was found to be around 5.1667minutes.

The estimation of Pioglitazone hydrochloride tablets was carried out by RP-HPLC using Mobile phase having a composition of 600 volumes of Methanol, 400 volumes of phosphate buffer. The pH was found to be 6. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Symmetry-Extend C_{18} (150 × 4.6mm, packed with 5µm). Flow rate of Mobile phase was 1.0ml/min. And all the Optimized chromatographic conditions are listed in table no.1

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Younglin HPLC with UV-detector)
Column	Symmetry-Extend C_{18} (150 × 4.6mm, packed with 5µm) is suitable
Mobile Phase*	Phosphate buffer: Methanol (40:60)
Flow rate	1.0ml/min
Detection	UV at 240nm
Injection volume	20µl
Temperature column	Ambient

System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates -7104.72, and tailing factor - 1.41. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.005% which shows that the method is repeatable.

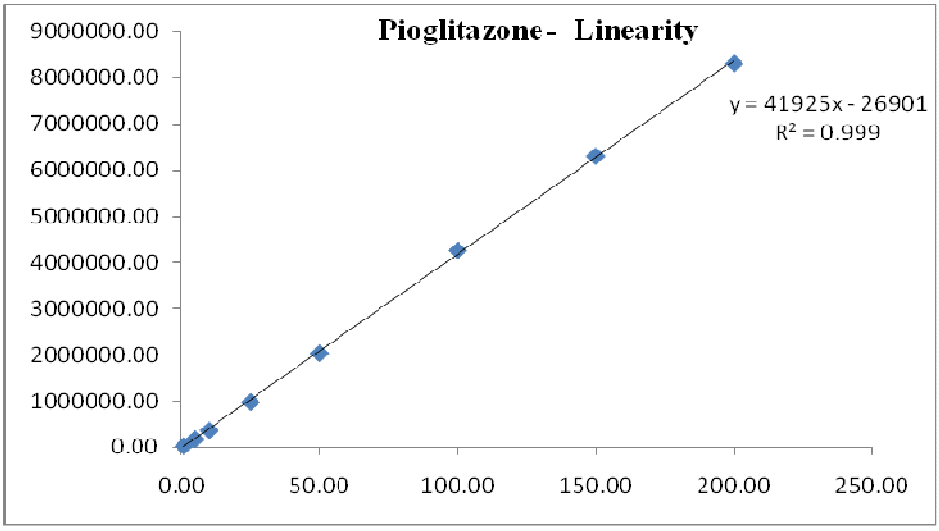

Fig. 2: Linearity of Pioglitazone Hcl

The acceptance criteria of Method Precision and injection Precision %RSD should be not more than 2.0% and the method show Method Precision 0.02% and injection Precision 0.005% which shows that the method is precise.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 1-200µg/ml of target concentration

for Pioglitazone hydrochloride standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) = 0.999, which shows that the method is capable of producing good response in UV-detector

The Accuracy limit is the % recovery should be in the range of 100.25 - 101.13%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy. And the results of all System suitability parameters are listed in table no.2

Table 2: System suitability parameters

Parameter	Glimepiride Hydrochloride
Calibration range (µg/ml)	1-200
Theoretical plates	7104.72
Tailing factor	1.41
Correlation Coefficient(r^2)	0.999
% Recovery	99.3% - 103.2%
System Suitability %RSD	0.005%
Method Precision %RSD	0.02%
Injection Precision %RSD	0.005%

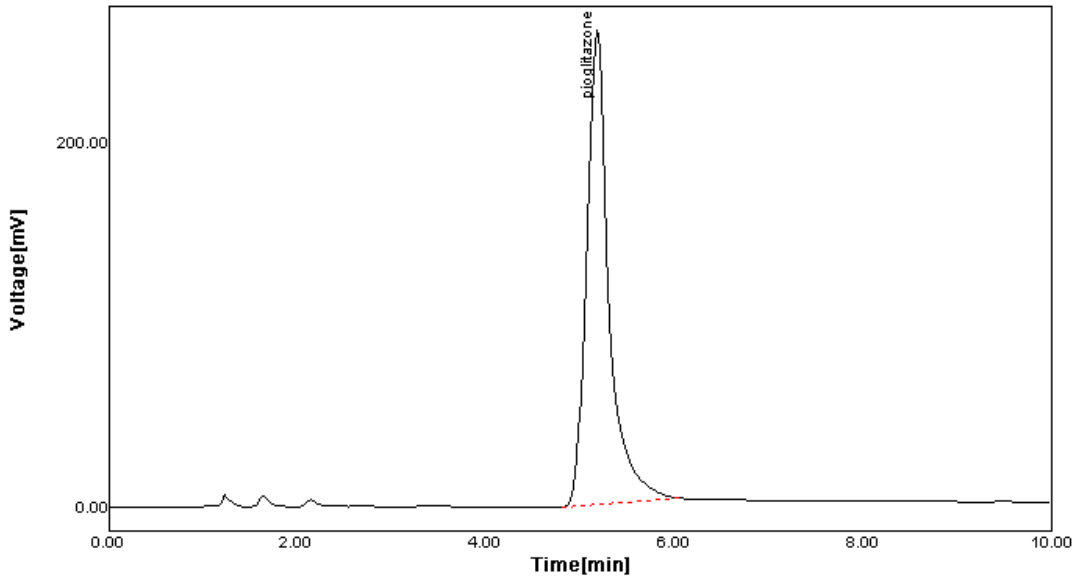

Fig. 3: Standard Chromatogram of Pioglitazone Hcl

CONCLUSION

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Pioglitazone hydrochloride is done by reverse phase HPLC. The mobile phase consists of buffer (400 volumes of Phosphate buffer, and 600 volumes of Methanol. The ratio pH was found to be 6.0. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes). The detection is carried out using PDA-Detector set at 240nm. The solutions are chromatographer at the constant flow rate of 1.0ml/min. The Retention time for Pioglitazone hydrochloride was around 5.1667minutes. Linearity range for Pioglitazone hydrochloride is 1 to 200µg/ml.

The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 100µg/ml. the quantitative results obtained is subjected to the statistical validation. The values of

RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 99.3% to 103.2% for Pioglitazone hydrochloride.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer's law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision.

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