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Research Article

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR DETERMINATION OF PIOGLITAZONE HCL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and precise RP-HPLC method was developed and validated for the determination of Pioglitazone hydrochloride in pharmaceutical dosage forms. Chromatography was carried out using C18 column (250x4.6mm), mixture of Buffer: acetonitrile (55:45%v/v) as the mobile phase at a flow rate 1.0 ml/min. The analyte was monitored using UV detector at 254 nm .The Retention time of the drug was 9.738min for Pioglitazone HCl. The proposed method was found to have linearity in the concentration range of 0.1-0.6 µg/ml with correlation coefficient of r^2=0.9999. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Pioglitazone HCL 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 Pioglitazone HCL in bulk and dosage forms.

Keywords: Pioglitazone HCL, RP-HPLC.

INTRODUCTION

Pioglitazone hydrochloride **Fig.1**, ((±)-5-{p-[2-(5-ethyl-2-pyridyl)ethoxy] benzyl}-2,4-thiazolidinedione hydrochloride) is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus (also known as non-insulin-dependent diabetes mellitus[1] (NIDDM) or adult-onset diabetes). Pioglitazone decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Currently, it is marketed under the trade name Actos®[2].

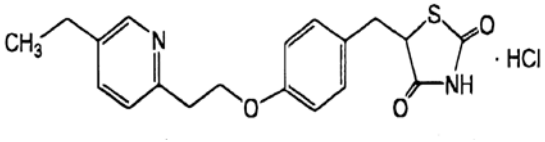

Fig.1: Structure of Pioglitazone HCL

As per literature survey few analytical methods have been reported for determining Pioglitazone hydrochloride in tablets [3-6].Yamashita determined Pioglitazone and its metabolites in human serum and urine[7]. The quantitative determination of Pioglitazone in human serum by direct-injection HPLC mass spectrometry and its application to a bioequivalence study has also been reported [2], Zhong and Lakings reported an assay method for Pioglitazone alone in dog plasma[8]. In the present study a simple reliable and reproducible RP-HPLC method was developed, validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines [9].

MATERIALS AND METHODS

Instrumentation

Analysis was performed using High Performance Liquid Chromatography system (HPLC) Waters 2695 model equipped with a UV-Visible detector. The output signal was monitored and processed using Empower software.

Chemicals and reagents

Pioglitazone HCL was obtained as a gift sample from PharmaZell R&D centre, India Pvt.Ltd. Ammonium acetate (AR grade), acetonitrile (HPLC grade) and acetic acid (AR grade).

Chromatographic conditions

Mobile phase consists of Acetate buffer, acetonitrile in the ratio 55:45v/v. Buffer was prepared by dissolving 1.54 g of ammonium acetate in 1000 ml water and pH adjusted to 4.6±0.05 with dilute acetic acid, Filtered through 0.45µ membrane filter. 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 30min with mobile phase flowing through the system. The eluents were monitored at 254nm.

Diluents: Mobile phase

Standard Preparation

Stock solution of Pioglitazone HCL was prepared by dissolving 100mg of Pioglitazone HCL in 50 ml volumetric flask add few drops of tetrahydrofuran to dissolve the drug and the volume is made up with mobile phase. Subsequent dilutions of this solution ranging from 0.1-0.6µg/ml were made with the mobile phase.

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 tetrahydrofuran 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.

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 Pioglitazone HCL in pharmaceutical dosage forms. The present method contains mobile phase Acetate buffer and acetonitrile in the ratio (55:45v/v) which was found to be the most suitable as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. Under the above conditions the retention time obtained for Pioglitazone HCL was 9.738 min. A model Chromatogram was shown in Fig. 2.

Linearity

The calibration curve for Pioglitazone HCL was drawn by plotting the mean peak area versus concentration yielded coefficient of regression r^2=0.9999 over a concentration range (100-600µg/ml) the representative linear regression equation for Pioglitazone HCL Y=10888 X+21293 as shown in Fig. 3.

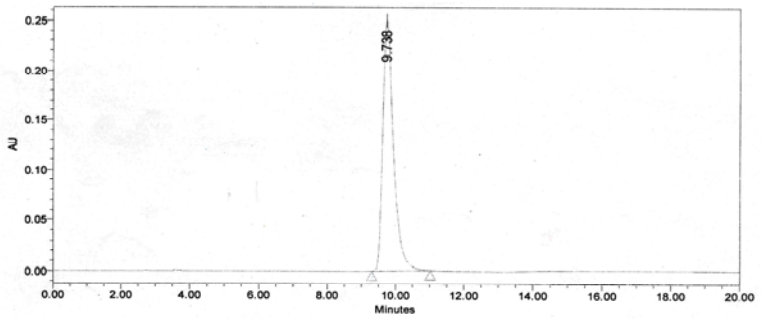

Fig. 2: Typical chromatogram for Pioglitazone HCL

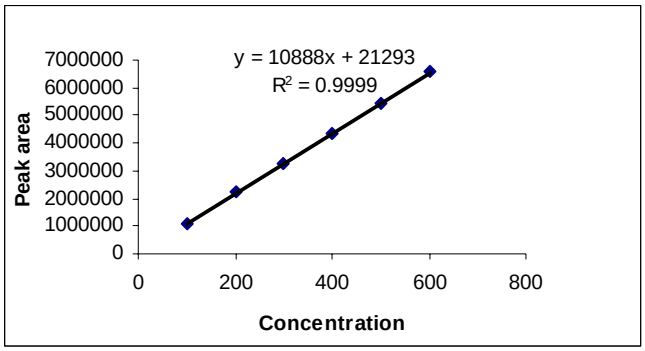

Fig. 3: Linearity graph of Pioglitazone hydrochloride

Accuracy

The accuracy of the proposed analytical method was determined by recovery experiments. The recovery studies were carried out at three different concentration levels in triplicate (50, 100 and 150%). The analyzed samples yielded high recovery values from the developed method .The % recovery results of the method are given in Table-1.

Table 1: Recovery study data

Amount of drug added (µg/ml)	Amount found(µg/ml) (N=3)	% Recovery(N=3)
25.06	24.96	99.61
50.11	50.12	100.03
75.23	75.27	100.19

Precision

The precision of the method for the determination of Pioglitazone HCL was studied using the parameters i.e. system precision, method precision and intermediate precision. System precision was determined by six replicate injections of standard solution injected in to the HPLC system. The relative standard deviation was less than 2%. Method precision was determined by the six individual sample preparations injected to the HPLC system. The relative standard deviation was less than 2%. Ruggedness of the method was determined by different analysts, different columns and different instruments on different days.

RSD was found below 2%. The results indicating that the developed HPLC method was found to be precise.

Robustness

The robustness of the method was studied by small changes in the method like altering the mobile phase pH, flow rate and changes in wavelength. It was observed that there were no changes in the chromatograms.

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and no.of theoretical plates were calculated. The results are given in Table-2.

Table 2: System suitability studies

S. No	Parameters	Results
1	Tailing factor	1.41
2	Theoretical plates	6794.61
3	Retention time	9.767
4	Purity 1 Angle	0.11
5	Purity 1 Threshold	0.31

The developed HPLC method in the present study has also been used to quantify Pioglitazone HCL in the tablet dosage forms. Pioglitazone HCL was quantified using the proposed analytical method and the results are given in Table-3.

Table 3: Analysis of Pioglitazone HCL in pharmaceutical formulations

Labeled amount (mg)	Observed amount (mg)	% Purity
15	14.81	98.73
30	30.13	100.43
45	44.96	102.18

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 Pioglitazone HCL in Tablet dosage forms. The method was duly validated by using required statistical parameters.

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