Available Online through
www.ijpbs.com

IJPBS |Volume 1| Issue 4 |OCT-DEC |2011|474-478

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETECTION OF PIOGLITAZONE HYDROCHLORIDE IN DOSAGE FORMS

D Adukondalu [1], P S Malathy[1], J Venkateshwar rao[1], Y Madhusudan Rao[2]*

[1]Talla Padmavathi college of pharmaceutical sciences, Warangal, A.P., India.

[2]University college of pharmaceutical sciences, K.U., Warangal, A.P., India.

***Corresponding Author Email:**

Research Article

RECEIVED ON 15-10-2011 **ACCEPTED ON 07-11-2011**

ABSTRACT

Objective of this work was to develop and validate a method for detection of Pioglitazone hydrochloride (Thiazolidinedione, very potent and effective anti diabetic agent) in tablet dosage forms by High performance liquid chromatography. The HPLC system consisting Waters analytical C18 column (46×250 mm), detected at 245 nm, sensitivity was 0.001 AUFS and flow rate was 1mL/min. The mobile phase composed of 10 mM phosphate buffer (pH 4.5) and Acetonitrile (50:50). Pioglitazone hydrochloride was extracted from tablet dosage forms and samples were spiked in to HPLC system. A linear relationship was observed in the range 10-750 ng/mL. The recovery of Pioglitazone hydrochloride was found to be more than 90%, Limit of quantification was 10ng/mL and Limit of detection was 2.5ng/mL. Thus the method so developed was found to be simple, rapid, specific, sensitive, repeatable and accurate.

KEYWORDS: *Pioglitazone hydrochloride, HPLC, Tablets.*

INTRODUCTION

Pioglitazone hydrochloride is thiazolidinedione is very potent synthetic peroxisome proliferators-activated receptor (PPAR)-γ inhibitor and effective anti diabetic agent. It exerts its glucose lowering effects by increasing insulin sensitivity in liver and peripheral tissue. [1, 2].This study describes simple method for determination of Pioglitazone hydrochloride in tablets by RP-HPLC with UV detection.

As per literature survey few analytical methods have been reported for determining pioglita one hydrochloride in tablets and biological samples. [3-7]

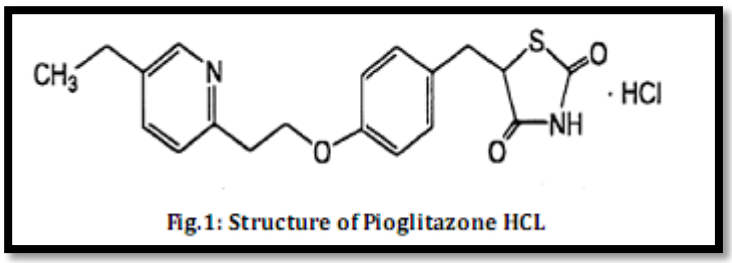


Fig.1: Structure of Pioglitazone HCL

OBJECTIVE

To estimate Pioglitazone hydrochloride in dosage forms and different biological samples various methods utilizing advanced, tedious and expensive techniques are available. We aimed to develop a simple, accurate, precise common and economic HPLC method for Pioglitazone hydrochloride estimation in tablets.

MATERIALS AND METHODS

Pioglitazone hydrochloride obtained from Matrix Pharmaceuticals, Hyderabad, A. P., India. Orthophosphoric acid, Acetonitrile and Methanol obtained from Merck specialities private limited Mumbai, Maharastra, India. All solvents used were of AR grade. Double distilled water was used throughout the procedure.

Preparation of standard and calibration standard solutions

Stock solutions were prepared by dissolving 100 mg of Pioglitazone hydrochloride in methanol of 100m.L. of volumetric flask to yield primary stock solution of 1mg/m.L. Secondary and working solutions were prepared by diluting with methanol. Calibration standards are prepared by spiking working standard solution into drug free plasma to yield concentration in the range of 10-750 ng/mL.

HPLC conditions

Waters HPLC unit consist of 515 HPLC pump, 20µL rheodyne loop, 2487 Dual λ Absorbance detector, Autochro 3000 software and Waters Analytical column C 18 46×250mm. The mobile phase composed of 10 mM phosphate buffer (pH 4.5) : Acetonitrile :: 50:50, detected at 245 nm, sensitivity is 0.001 AUFS and flow rate is 1mL/min.A linear relationship between concentration and peak area ratios of Pioglitazone hydrochloride (R.T. 6.3 min) was observed in the range 10-750 ng/mL.

Prepartion of sample solutions

Pioglitazone hydrochloride tablets (Pioglit, sun pharma) purchased from local market and ten tablets weighed and powderd in a mortar and equivalent weight of 15 and 30 mg Pioglitazone hydrochloride powder transefered separately to a separating funnel methanol was added, vigorously shaked for 30 mins, sonicated for 15 min and then filtered through 0.22 µm filter and from this three different concentrations were prepared and injected to HPLC column and method validated as per guidelines.[8, 9]

RESULTS AND DISCUSSION

Linearity

Linearity was observed for Pioglitazone hydrochloride concentrations of 10, 25, 50, 125, 250, 500, and 750ng/mL. The calibration curve was plotted by taking concentration on x-axis and Peak height ratios of drug and internal standard on y-axis. Calibration curve was plotted and equation was determined as $y=1.5421x + 68.63$, R^2 is 0.9921 and sample chromatograms represented in **Fig 2 & 3** and calibration curve represented in **Fig 4.**

Fig 2: Typical Chromatogram of Pioglitazone hydrochloride in Methanol

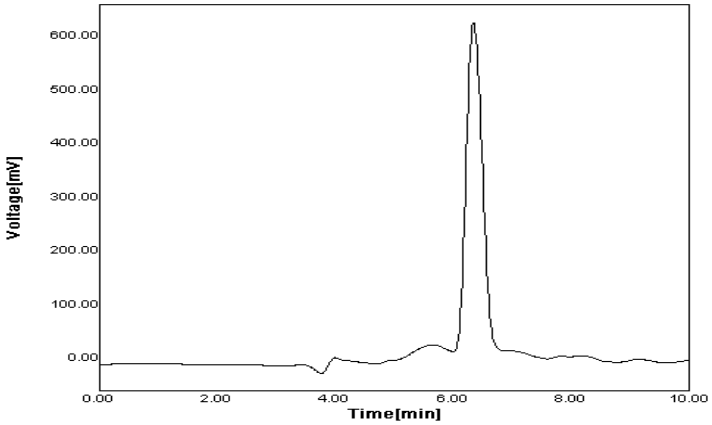


Fig 3: Typical chromatogram of Pioglitazone hydrochloride in Tablets.

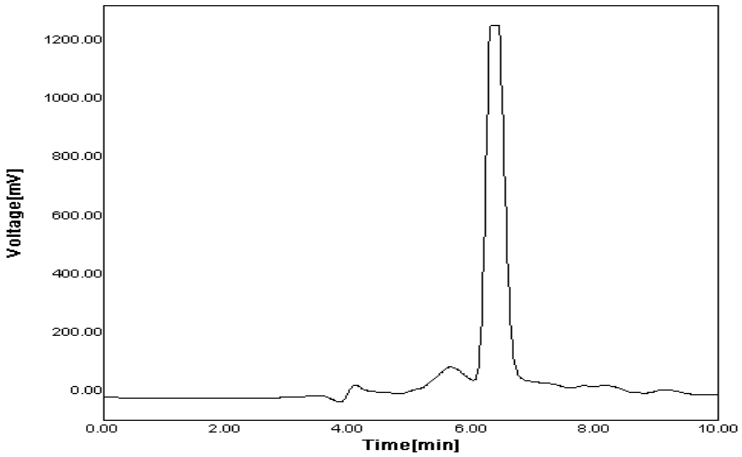
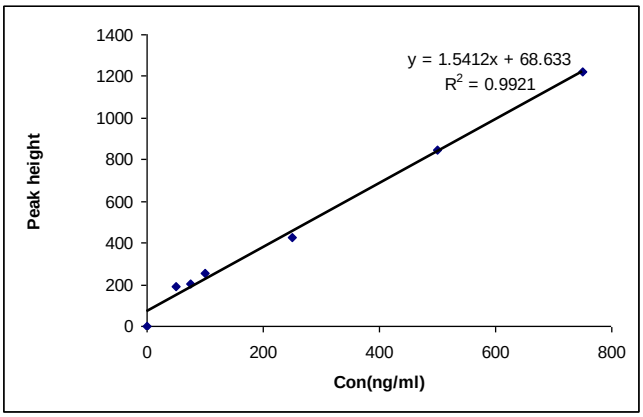


Fig 4: Calibration curve of Pioglitazone hydrochloride



LOD and LOQ

Limit of detection was determined by taking signal to noise ratio more than 3. Limit of quantification was determined by measuring the RSD of low concentration samples which was less than 20%. LOQ was 10ng/mL and LOD was 2.5ng/mL.

Accuracy and precision

Accuracy was calculated by comparing the peak heights of methanol solution with extracted sample containing different levels of drug concentrations. Precision was determined by calculating inter day and intraday variation for different levels of drug concentration. Accuracy of the method represented in recovery terms as 91-114%.

Intraday and inter day coefficient of variation is less than 10% and recovery studies results represented in **Table 1.**

Table 1: Analysis of Pioglitazone hydrochloride in pharmaceutical formulations

Labeled amount (mg)	Observed amount (mg)	% Purity
15	14.5	96.6
30	31	103.3

CONCLUSION

The method established for Pioglitazone hydrochloride detection in tablet dosage forms was simple extraction procedure, accurate, precise, U.V. detection, good LOD, LOQ to determine required drug levels and method applied suitably.

REFERENCES

1. J.Patel, E. Miller, R.Patwardhan, Diabetes 47(Supp .l) (1998) A17.
2. Unitedstates pharmacopeia, United states pharmacopeial convention, Rockville, 1995, 1982-4.
3. T Radha Krishna, J Satyanarayana, A Satyanarayana, Journal of pharmaceutical and biomedical analysis 2002, 29, 873-880.
4. Sayed S, Thomas A, Kotapalli L. RP-HPLC method development for determination of Pioglitazonehydrochloride from tablets. Journal of pharmacy research 2009; 2:1479-1480.
5. Sane RT, Menon SN, Inamdar S, Mote M, Gundi G. Simultaneous Determination of Pioglitazonehydrochloride and Glimepiride by High-Performance Liquid Chromatography. Chromatographia 2004; 59:451-453.
6. Radhakrishna T, Sreenivas Rao D, OmReddy G. Determination of Pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC methods.
7. J.Pharm.Biomed.Anal 2002; 29:593-607.
8. International conference on harmonization validation of analytical procedures methodology Q 2B ICH Harmonized tripartite guidelines, Adopted 6th Nov, 1996.
9. D Srinivasulu, B S Sastry, G Omprakash, Development and validation of new RP-HPLC Method for determination of Pioglitazone hydrochloride in Pharmaceutical Dosage forms, International Journal of chemistry research, 1 (1), 2010, 18-20.

*** Corresponding Author**
D.Adukondalu
Asst.Professor,
Talla Padmavathi College Of Pharmacy
Orus, Warangal, Andhra Pradesh