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A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF PIOGLITAZONE IN PHARMACEUTICAL DOSAGE FORMS

*A. Chandan Reddy, V. Vijayakumar, R. Sandeep Teja, P. Snehith, R. Karthik and G. N. V. Chandra Sekhar Reddy

Analytical Research and Development Center, PharmaTrain, Balajinagar, Kukatpally, Hyderabad-72

***Corresponding author e-mail:** chandurez@gmail.com and vijju.dreaming@gmail.com

ABSTRACT

A simple and rapid RP-HPLC method was developed for quantification of pioglitazone in tablet dosage form. The chromatography system used a reversed phase C_8 column with dual wavelength absorbance detection at 267 nm. The mobile phase consisted of acetonitrile and phosphate buffer (pH adjusted to 4.5 using ortho phosphoric acid) in the ratio of 60:40 % v/v at flow rate of 0.8 mL/min. The linearity range was found to be 20-60 µg/mL. The method was validated and it was concluded that the developed method was accurate, sensitive, precise, robust and useful for the quality control of pioglitazone in pharmaceutical preparations.

Keywords: Pioglitazone, RP-HPLC and Validation

INTRODUCTION

Pioglitazone hydrochloride (PIO) [(±)-5-[[4-[2-(5-ethyl-2- pyridinyl) ethoxy] phenyl] methyl]-2,4-] thiazolidine-dione monohydrochloride is an oral anti-hyperglycemic agent which acts primarily by decreasing insulin resistance. It is used in the treatment of type-II diabetes mellitus (2) 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]. The literature survey reveals that chromatographic methods were reported for determination of Pioglitazone and its metabolites, inhuman plasma [3-5], human serum [6,7], and urine [8] and in pharmaceutical formulations [10, 11]. The main purpose of this study is to develop a simple, rapid, accurate, linear, sensitive, rugged and reproducible HPLC method for the determination of PIO. The developed HPLC method was validated with respect to linearity, accuracy, precision, sensitivity, and robustness.

MATERIALS AND METHODS

Reagents and chemicals: PIO was generous gift from Suven Life Sciences Limited, Hyderabad (INDIA). PIO tablets containing 15 mg of active substance (DEPEL and CLOZE-BG) were obtained from commercial market and used within their self life period. HPLC grade acetonitrile, ortho phosphoric acid and potassium dihydrogen phosphate were obtained from Rankem, New Delhi, India. High purity deionized water was obtained from a Millipore, Milli-Q (Bedford) purification system.

Instrumentation: A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was XTerra symmetry C_8 (150×4.6 mm, 3.5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Chromatographic conditions*:* The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of acetonitrile and phosphate buffer (pH 4.5, pH adjusted with ortho phosphoric acid) in a ratio of 60:40 v/v. The analysis was performed at ambient temperature using a flow rate of 0.8 mL/min with a run time of 7 min. The eluent was monitored using DAD at wavelength of 267 nm. The mobile phase was filtered through 0.45 µm micron filter prior to use.

Preparation of stock and standard solutions*:* A stock solution of PIO (1000 µg/mL) was prepared by accurately weighed 10 mg of PIO reference standard into 10 mL volumetric flask and dissolved in 5 mL acetonitrile and volume was made up to the mark with acetonitrile. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solutions of PIO ware transferred using A-grade bulb pipettes into 100 mL volumetric flasks and solutions were made up to the mark with mobile phase to give the final concentrations of 20-60 µg/mL.

Estimation of PIOmitriptan from tablet dosage form*:* To determine the content of PIO in tablets (label claim: 15 mg), 20 tablets were taken and contents were weighed and mixed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 mL volumetric flask and was dissolved in 25 mL of acetonitrile and volume was made up to the mark with acetonitrile. The flask was sonicated for 25 min to affect complete dissolution.

The solution was filtered through a 0.45 µm micro filter. Suitable aliquot of the filtered solution was transferred into a 100 mL volumetric flask and made up to the volume with mobile phase to yield the concentration of 40µg/mL. The experiments were performed six times under the chromatographic conditions described above. The peak areas were measured at 267 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation [12]

Linearity: By appropriate aliquots of the standard PIO solution with mobile phase, five working solutions ranging between 20-60 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of PIO to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of PIO to which known amounts of standard PIO corresponding to 50, 100 and 150% of label claim were added. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of PIO at concentration of 20, 30 and 40µg/mL. Determinations were performed with three replicates on the same day as well as on three consequent days. The reproducibility of the method was checked by determining precision on a same instrument, analysis being performed by another person in same laboratory. It was analyzing the samples of PIO at different concentration (30, 40, 50 µg/mL) were determined in triplicate and calculate the amount of drug present in the sample.

Limit of detection and limit of quantification: Limit of detection (LOD) and limit of quantification (LOD) were calculated based on the ICH guidelines.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 0.8 to 0.7 mL/min and 0.9 mL/min. The organic strength of the mobile phase was varied by ±2%.

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20 µL of the working standard solution containing 20µg/mL were used for the evaluation of the system suitability parameters like tailing factor, number of theoretical plates and retention time.

RESULTS AND DISCUSSIONS

A RP-HPLC was proposed as a suitable method for the quantification of PIO in tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate were made on the basis of peak shape, baseline drift, time required for analysis, economical and the mobile phase consisted of acetonitrile and phosphate buffer (pH 4.5, adjusted pH with ortho phosphoric acid) in the ratio of 60:40 v/v at flow rate of 0.8 mL/min and analyzed at 267 nm. The retention time observed (3.373) allows a rapid determination of the drug. In

Figure 1, a typical chromatogram obtained under these conditions is shown. The calibration plot of peak area against concentration was linear in the range of 20-60 µg/mL. Calibration data, with their % relative standard deviation (%RSD) and linear regression equation are listed in Table 1. The range of reliable quantification was set at 20-60 µg/mL as no significant difference was observed in the slope of the standard curve in this range. The linear regression data for the calibration curve is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance. The LOD and LOQ were determined based on a signal-to-noise ratios and were based on analytical responses of 3 and 10 times the background noise, respectively. The LOD was found to be 0.05µg/mL. The LOQ was found to be 0.18µg/mL.

The accuracy was assessed from three replicates containing concentration of 20, 30 and 40µg/mL. The recovery of the method, determined by spiking a previously analyzed test solution with addition of standard PIO solution, was found to be in the range of 98.43-99.97%. The values of % recovery and %RSD are listed in Table 2, indicates that the method is accurate.

Precision of the method was measured in accordance with ICH guidelines. Repeatability of the method was determined as intra-day variation while intermediate precision was determined by measuring inter-day variation for triplicate determination of PIO at three different concentrations. The results of the determination of repeatability and intermediate precision are listed in Table 3. The low %RSD values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The assay values and low %RSD values indicate that the method is reproducible.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors consider were: variations in the flow rate (±0.1) and percentage of acetonitrile (±2%). There were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing that the method is robust. The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 4, showing that the parameters are within the suitable range.

The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 5. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. The PIO content was found to be 98.93% and 99.13 for DEPEL and CLOZE-BG, respectively. The low %RSD indicated the suitability of this method for routine analysis of PIO in pharmaceutical dosage forms, shown in Table 5.

CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of PIO from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures and low t_R. All these factors make this method suitable for quantification of PIO in tablet dosage forms. The method can be successfully used for routine analysis of PIO in bulk drugs and pharmaceutical dosage forms without interference.

Table 1: Linearity regression data for the calibration plot of PIO

Analyte	Conc. (µg/mL)	Mean area ±SD (n=3)	RSD (%)	Linear regression equation
PIO	20	4497565±58183	1.294	y = 200155x + 539701
	30	6608270±7703	0.073	
	40	8504261±126061	1.482	r^2=0.9996
	50	10619844±7703	0.073	
	60	12499515±210015	1.68	

Table 2: Results of recovery studies

Analyte	Amount (%) of drug added to analyte	%Mean recovery ± SD (n=3)	RSD (%)
PIO	50	98.77±0.501	0.507
	100	99.97±0.849	0.85
	150	98.43±0.522	0.53

Table 3: Intra-day and Inter-day precision of the method

Analyte	Conc. (µg/mL)	Repeatability precision (intra-day)*	Intermediate precision (inter-day)*
PIO	20	1.076	0.827
	30	0.865	0.836
	40	0.462	0.428

%RSD*

Table 4: Results of system suitability tests

Parameters	Results of PIO
Retention time (min)	3.373
Tailing factor	1.15
Theoretical plates (N)	4069

Table 5: Analysis of pioglitazone in tablets

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)	SEM
DEPEL	15	98.93±0.879	0.889	0.359
LCOZE-BG	15	99.13±0.505	0.51	0.2062

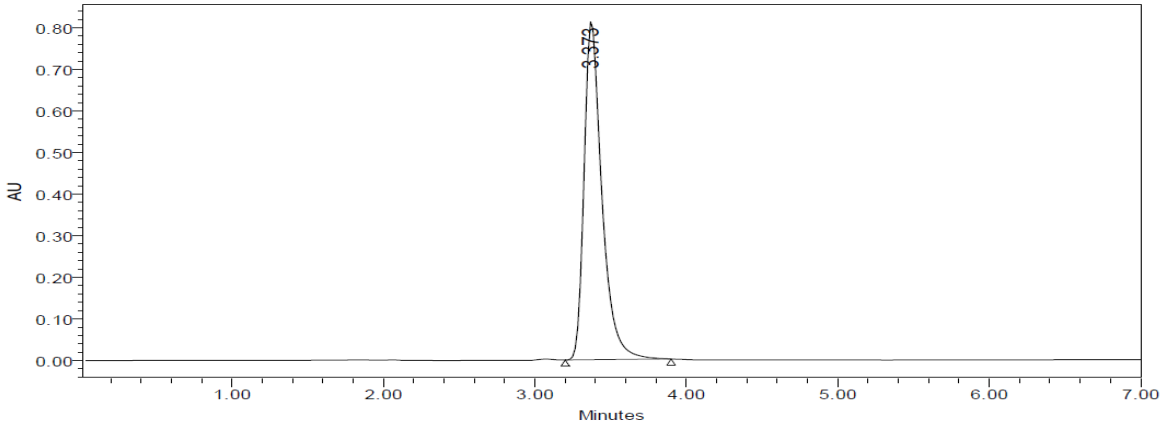

Figure 1: A typical chromatogram of PIO

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