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Novel UV and visible spectrophotometric methods for the estimation of pioglitazone hydrochloride in bulk drug and pharmaceutical dosage forms

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ABSTRACT

Two simple, selective, rapid, accurate, precise and cost-effective spectrophotometric methods for the determination of Pioglitazone Hydrochloride (PIO) in bulk drug and in tablets have been developed and validated. First method (method A) is based on the measurement of absorbance of PIO in methanol: 0.1M HCl: water (1:3:1) by cosolvancy technique at 269.2 nm. The second method (method B) is based on the measurement of absorbance of bluish green coloured chromogen complex at 765 nm which is formed by reaction of PIO with ferric chloride and potassium ferricyanide (redox technique). Beer's law was obeyed over the ranges 10-45, 50-350 µg/mL for method A and method B respectively. The limit of detection (LOD), limit of quantification (LOQ) and sandell's sensitivity values are reported. All the methods were validated in accordance with ICH guidelines. The results of the analysis have been validated statistically. The methods have been successfully applied in the analysis of marketed formulations. No significant interference was observed from the excipients commonly used as pharmaceutical aids with the assay procedure.

KEYWORDS

Spectrophotometric analysis;
Pioglitazone hydrochloride;
Estimation;
Cosolvancy;
Redox method,

INTRODUCTION

Pioglitazone Hydrochloride (PIO) belongs to the class of thiazolidinediones. Chemically it is (±)-5-[p-[2-(5-ethyl-2-pyridyl) ethoxy] benzyl]-2, 4-thiazolidinedione monohydrochloride[1]. PIO is a potent and highly selective agonist for the peroxisome proliferator activated receptor-gamma (PPAR). It improves insulin response to target cells with increasing the pancreatic secretion of insulin. Literature survey revealed HPLC[4,5,7], HPTLC[8], Colorimetric[6] and UV methods in bulk drug and pharmaceutical dosage forms. The chromatographic techniques were the most widely used. Although, the procedures are specific, most of the described methods are time consuming. On the other hand, the reported spectrophotometric methods suffer from one or the other disadvantage such as poor sensitivity, scrupulous control of experimental variables and special equipment.

The present study reports the development, validation and estimation of PIO pure form and its solid dosage forms. The developed methods were found to posses several advantages in terms of sensitivity, speed and cost-effectiveness compared to the reported

spectrophotometric methods.

EXPERIMENTAL

Instrument

Double-beam Shimadzu UV-Visible Spectrophotometer 1800, with spectral bandwidth of 1.0 nm, wavelength accuracy ± 0.1 nm and a pair of 1cm path length matched quartz cells were used to measure absorbance of the resulting solution.

Materials

Standard gift sample of Pioglitazone Hydrochloride was provided by

M/s. Aarti Drugs Ltd., Mumbai, India. The pharmaceutical dosage forms (tablets) - Pioglar-Ranbaxy (30 mg), PATH – Lupin (30 mg), were purchased from local market.

Reagents and solutions

All the chemicals used were of analytical reagent grade. Distilled water was used throughout the study.

(a) Hydrochloric acid (0.1 M)

Prepared by successive dilutions of appropriate volume of concentrated acid (SD Fine Chemicals, Mumbai, India) in water.

(b) Hydrochloric acid (1M)

Prepared by successive dilutions of appropriate volume of concentrated acid (SD Fine Chemicals, Mumbai, India) in water.

(c) Potassium ferricyanide (0.3%)

Potassium ferricyanide (0.3%) was prepared by dissolving 0.3 gms of potassium ferricyanide in 100 ml of water.

(d) Ferric chloride (3%)

Ferric chloride (3%) was prepared by dissolving 3 gms of ferric chloride in 100 ml of water.

General procedures

(a) Method A

10 mg of PIO pure drug was accurately weighed and dissolved in minimum quantity of methanol: 0.1MHCl: water (1:3:1) and diluted to 10 ml with same solvent. 1.0 ml of this solution was again diluted to 10 ml with the same solvent. Suitable aliquots of the standard solution of PIO (1.0 - 4.5 ml) were taken in 10 ml volumetric flasks. The volume was then made upto the mark with methanol:0.1MHCl:water (1:3:1) to prepare a series of standard solutions containing 10 - 45 µg/ml. Absorbance was measured at 269.2 nm against blank Figure 1. Linearity was checked, the concentrations ranging from 10 - 45 µg/ml obeys Beer's law[2] Figure 2.

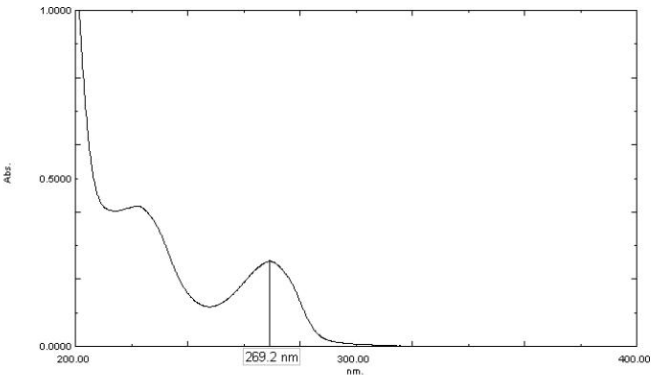

Figure 1 : Absorption spectra of PIO in methanol: 0.1M HCl: water (1:3:1)

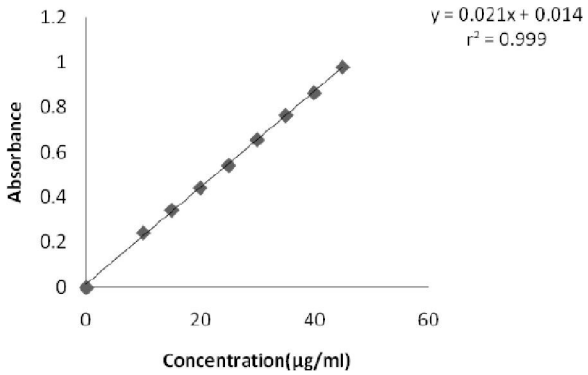

Figure 2 : Calibration curve (Linearity) of pioglitazone hydrochloride in methanol: 0.1NHCl: water (1:3:1) at 269.2 nm

(b) Method B

10 mg of PIO pure drug was accurately weighed and dissolved in minimum quantity of methanol and diluted to 10 ml with same solvent. 1.0 ml of this solution was again diluted to 10 ml with the same solvent. Suitable aliquots of the standard solution of PIO (0.5-3.5 ml) were taken in 10 ml volumetric flasks and to these solutions 3% ferric chloride (0.5 ml), 0.3% potassium ferricyanide (0.5 ml) and 1M hydrochloric acid (0.5 ml) were added. The volume was then made upto the mark with water to prepare a series of standard solutions containing 50-350 µg/ml. Absorbance was measured at 765nm against blank Figure 3. Linearity was checked,

the concentrations ranging from 50-350 µg/ml obeys Beer's law, Figure 4.

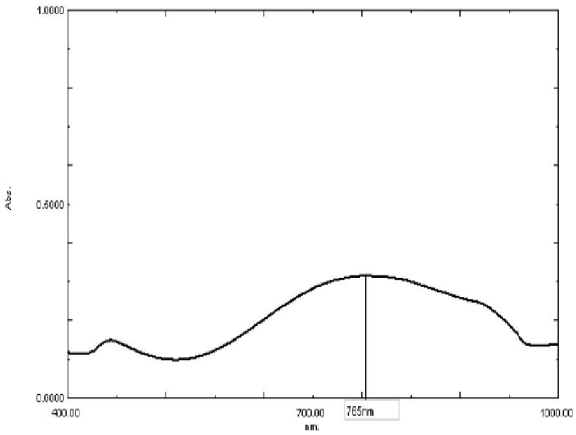

Figure 3 : Absorption spectra of PIO in 3% ferric chloride, 0.3% potassium ferricyanide and 1M hydrochloric acid

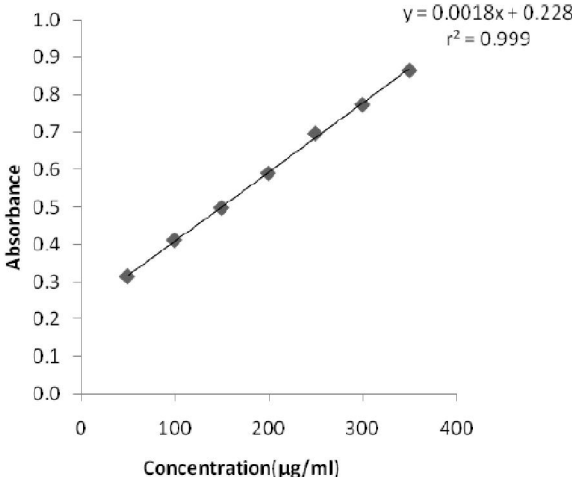

Figure 4 : Calibration curve (Linearity) of pioglitazone hydrochloride at 667 nm with potassium ferricyanide

In both the methods, calibration curves were prepared and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using Beer's law data.

Procedure for estimation of PIO in tablets

(a) Method A

Twenty tablets (of same respective batch number) of two pharmaceutical companies Pioglar (Ranbaxy), PATH (Lupin) were accurately weighed and powdered. Weight of powdered tablets equivalent to 10 mg of drug was taken in few ml of methanol:0.1MHCl:water (1:3:1) and vigorously shaken for 10 minutes, filtered through whatmann filter paper No.41 and made up to 10 ml. 1 ml of the above solution was diluted to 10 ml with methanol: 0.1MHCl: water (1:3:1).

3.0 ml of above solution was transferred into a series of 10 ml volumetric flasks and the final volume was brought to 10 ml with methanol: 0.1MHCl: water (1:3:1). The absorbance was measured at 269.2 nm against methanol: 0.1MHCl: water (1:3:1) as blank and the amount of PIO present in the sample solution was calculated. The experiment was repeated six times for each brand of tablets. Drug content in each brand of tablet was calculated and the results are given in TABLE 1.

(b) Method B

The quantity equivalent to 10 mg of active ingredient was dissolved in methanol and the volume was made upto 10 ml to get a stock solution. Subsequent dilutions of this solution were made, to it aqueous solution of 3% ferric chloride (0.5 ml), 0.3% potassium ferricyanide in distilled water (0.5 ml) and 1M hydrochloric acid (0.5 ml) were added. The solutions were kept aside for 10 minutes and stirred occasionally. The solutions were made upto the mark with distilled water and the absorbance of the bluish green coloured chromogen was measured at 765 nm against the corresponding reagent blank. Drug content in each brand of tablet was calculated and the results are given in TABLE 1.

TABLE 1 : Results of analysis of PIO tablet formulations

Method	Tablet formulation	Label claim per tablet (mg)	% Label claim estimated (mean ± standard deviation)*	% RSD
A	I	30	99.18 ± 0.1803	0.181
	II	30	99.73 ± 0.1479	0.148
B	I	30	99.96 ± 0.2902	0.2901
	II	30	101.26 ± 1.5885	1.5687

***Average of six determinations; %RSD = percentage relative standard deviation**

The content of PIO in method A and method B was calculated by using the below formula.

Content of Pioglitazone Hydrochloride in average weight of each tablet =

$$\frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \left[\frac{\text{Standard dilution}}{\text{Test dilution}}\right] \times \text{Average weight of tablet}$$

METHOD VALIDATION[3]

Accuracy

Accuracy of the methods was determined by

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recovery experiments. To the formulation, the reference standard of the respective drug was added at the level of 80,100,120% to the formulation and further diluted by procedure as followed in the estimation of formulation. The concentration of the drug present in the resulting sample solution was determined by using assay method TABLE 2.

Precision

The assays described under general procedures were repeated six times within the day to determine the repeatability (intraday precision) and six times on different days to determine the intermediate precision (interday precision) of the methods. The results of this study were summarized in TABLE 3.

TABLE 2 : Results of accuracy (Recovery) studies

	Method A					Method B			
Tablets Used	% level of recovery	Amount of the drug in tablet Powder (mg)	PIO pure drug added (mg)	% recovery estimated	% RSD*	Amount of the drug in tablet powder (mg)	PIO Pure drug added (mg)	% recovery estimated	% RSD*
I (Pioglar)	80	30	24	100.11	0.63	15	12	100.33	0.50
	100	30	30	100.01	0.34	15	15	100.03	0.43
	120	30	36	100.09	0.58	15	18	100.21	0.87
II (PATH)	80	30	24	100.11	0.94	15	12	99.88	0.56
	100	30	30	100.18	0.16	15	15	99.93	0.46
	120	30	36	100.12	0.11	15	18	99.93	0.09

*Average of six determinations; %RSD = percentage relative standard deviation

TABLE 3 : Results of precision

Method A						
Concentration (µg/ml)	Intra-day Precision (n=6)			Inter-day Precision (n=6)		
	Amount Found (µg/ml)	SD	%RSD*	Amount found (µg/ml)	SD	%RSD*
10	10.92	0.138	1.263	10.83	0.028	0.258
20	20.33	0.05	0.245	20.40	0.023	0.112
30	30.60	0.051	0.166	30.64	0.057	0.186
Method B						
Concentration (µg/ml)	Intraday Precision (n=6)			Inter-day Precision (n=6)		
	Amount found (µg/ml)	SD	%RSD*	Amount found (µg/ml)	SD	%RSD*
100	101.47	0.849	0.836	101.22	0.856	0.845
200	201.10	2.225	1.106	201.10	0.555	0.275
300	301.17	0.453	0.150	300.89	0.320	0.106

n = number of measurements; SD = standard deviation; %RSD = percentage relative standard deviation; *Average of six determinations

Linearity

From the standard stock solutions, suitably mixed standard solutions were prepared. The solutions were examined by the assay procedure. The calibration curves were plotted using concentration Vs absorbance of the standard solution. The slope and correlation coefficient values were calculated. The correlation coefficient of PIO in method A and method B were found to be 0.999 at 269.2nm and 765nm respectively. The calibration curve was plotted using concentration Vs absorbance of the standard solution. The calibration graph showed that a linear response was obtained over the range of concentrations used in the assay procedure. These data clearly demonstrates that the developed method has adequate sensitivity to the concentration of the analytes in the sample.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were separately determined and reported, based on the calibration curve of standard solution. The relative standard deviation of the regression line or the standard deviation of y – intercepts of regression lines may be used to calculate LOD and LOQ. The LOD and LOQ of the developed methods were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). The LOQ is the lowest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The values were summarized in TABLE 4.

TABLE 4 : Results of optical characteristics

Parameter	Method A	Method B
λ_{max}	269.2nm	765nm
Beer's law limit (µg/ml)	10-45	50-350
Regression equation (Y)	0.021x +0.014	0.025x+0.006
Slope (m)	0.021	0.025
Intercept (c)	0.014	0.006
Correlation coefficient (r^2)	0.999	0.999
Limit of Detection (µg/ml)	0.3259	0.9166
Limit of Quantification (µg/ml)	0.9876	2.7777
Molar absorptivity (mol/lit/cm)	$0.9591x\ 10^4$	$0.9677x10^4$
Sandell's sensitivity (µg/ml 0.001 abs unit)	0.046128	0.5466

RESULTS AND DISSCUSION

The solubility of PIO was determined in a variety of solvents using Schefter and Higuchi method. The proposed methods are simple and precise and do not suffer from any interference due to common excipients of tablets. Methods were validated in terms of accuracy, precision, LOD, LOQ and linearity. The accuracy of the methods were proved by performing recovery studies in the commercially available formulations. Values greater than 99 % indicate that proposed methods are accurate for the analysis of drug. The results for raw material and formulations in the proposed methods were found to be satisfactory. In an over view the results indicate that the methods are precise enough for the analysis of the drug.

Method A

PIO is insoluble in water hence cosolvancy technique has been employed. In this technique, water insoluble drugs can be made soluble by combining two (or) more solvents. Stability has been maintained by adding an acid.

Method B

PIO exhibits reducing property due to the presence of functional moieties (one or more) vulnerable to oxidation selectively with oxidizing agents such as ferric chloride. Under controlled experimental conditions when treated with known excess of oxidant, PIO undergoes oxidation, giving products of oxidation (inclusive of reduced form of oxidant Fe (II) from Fe (III)) besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either reduced oxidant or reduced form of oxidant formed. The reduced form of Fe (III) i.e., Fe (II) has a tendency to give a blue green coloured complex on treatment with potassium ferricyanide. The absorbance of bluish green coloured complex formed was measured at 765 nm against the corresponding reagent blank.

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