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[Research article]

Analytical method Development and Validation for the estimation of Pioglitazone hydrochloride in Bulk and Tablet dosage form by UV_Spectroscopy

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ABSTRACT

This paper describes the analytical method suitable for validation of Pioglitazone hydrochloride by UV Spectrophotometric method. The method utilized UV spectroscopy and the solvent system was consists of 6 N Glacial acetic acid at wave length 270 nm. Validation experiments were performed to demonstrate Specificity, Precision, Linearity, Accuracy, ruggedness. The method was linear over the concentration range of 10-50 µg/ml. The Proposed method was simple, sensitive & reliable with good Precise, Accurate, and Reproducible and rapid for the determination of Pioglitazone. The commercial formulations are estimated without interference. Hence this method can be used for routine determination of Pioglitazone hydrochloride in bulk and their pharmaceutical dosage forms.

Key words: UV-Spectrophotometry, Pioglitazone hydrochloride, Pharmaceutical dosage form.

INTRODUCTION

Pioglitazone hydrochloride is an oral anti-hyperglycemic agent which is used in the treatment of type 2diabetes mellitus. This type of diabetes mellitus is also known as non-insulin-dependent diabetes mellitus (NIDDM). This syndrome is characterized by hyper glycemia resulting from abnormalities in insulin secretion, action or both Systematic (IUPAC) Name: (±)-5-{[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl}-2,4-thiazolidinedione mono hydrochloride[1,2]. The aim of the present study was to develop and validate a simple, UV Spectroscopic method for the determination of Pioglitazone hydrochloride in bulk and tablets. The developed method was validated using ICH guidelines for validation (ICH,1995) [2]

Materials and Methods

Instrument

Absorption spectral measurements were carried out with a UV – Visible spectrophotometer (Analytical technologies model spectro 2060 plus version 5) was employed with spectral band width of 5 nm and wavelength accuracy of 0.3nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells).

Chemicals

Pioglitazone hydrochloride pure drug was supplied by Hetero Labs, India as gift sample and used as such. Spectroscopy graded water and analytical reagent grade glacial acetic acid were used.

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Preparation of standard stock solution

Standard solution of pioglitazone hydrochloride was prepared by dissolving 10 mg of Pioglitazone hydrochloride in 10ml of mobile phase (6N GAA) to get concentration 1000µg/ml. Different aliquots of above solution in the range 0.1 to 0.5 ml were transferred into series of 10ml volumetric flask and volume made up to the mark with water to obtain the concentrations 10 to 50µg/ml. scanning ranges was finalized for study and solutions were scanned on spectrophotometer in the UV range of 200-400nm.

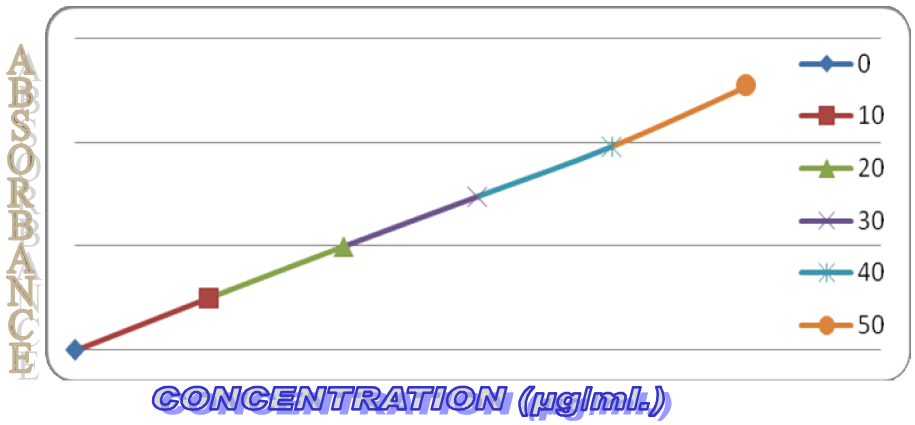
Preliminary solubility studies of drugs

A small quantity of standard drug was dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, and in various buffer solutions. By the solubility studies we determined that the drug was dissolved in 6N glacial acetic acid, hence this solvent system was used in the present study.

Determination of λ max

From the stock solutions, a working standard was prepared. The absorption spectrum for pioglitazone hydrochloride, the absorption spectrum was recorded using 10µg/ml solution and the maximum absorption was found to be 270nm. The Calibration curves were prepared for Pioglitazone the concentration range of 10-50 µg/ml at selected wave lengths by diluting aliquot portions of stock solution of each drug. The plots of Beer's law limit are shown in Fig.1.

Fig.1 Calibration curve of Pioglitazone hydrochloride



Preparation of Sample solution

Sample label claim 30 mg. The average weight was determined with 20 tablets, which were grounded in a mortar until fine powder. Accurately weighed amount of powder equivalent to 10 mg of Pioglitazone hydrochloride was quantitatively transferred to a 10 ml calibrated volumetric flask with the mobile phase (6N GAA). The volume was made up to mark, shake for 15 min and filter the sample solution using What man filter paper No-1. From above solution 1ml was transferred to 10 ml calibrated volumetric flask and made up to mark with the aid of 6 N GAA to obtain the concentration 100 µg /ml. From above solution 0.3 ml was transferred to 10 ml calibrated volumetric flask and made up to mark with the aid of 6N GAA to obtain the concentration 30 µg/ml. Then the solution was scanned from 200-400 nm.

METHOD VALIDATION

The method was validated with reference to linearity, accuracy, precision, and specificity, ruggedness. [5]

Linearity

Linearity was performed by taking aliquots of 0.1, 0.2, 0.3, 0.4 and 0.5 ml from stock solution (1mg/ml) in 10ml volumetric flasks and diluted up to the mark with the (6N GAA) such that the final concentration of Pioglitazone in the range of 10 to50 µg/ml. Under the experimental conditions described the graphs obtained by plotting concentration (µg/ml) vs absorbance. The observations and calibration curve is shown in Table 1 and Fig.1

TABLE: 1 OPTICAL CHARACTERISTIC AND LINEARITY DATA

Parameters	Observations
λmax for Pioglitazone hydrochloride	270 nm
Beer's law limits	10-50 µg/ ml
Correlation coefficient	0.999
Regression equation (Y=mx+c)	Y= -0.009+0.025x
Intercept(a)	-0.0094
Slope(b)	0.02528
Molar absorptivity	9888.67L/Mole/Cm
Sandell sensitivity	0.039735 µg/ ml

Accuracy

The accuracy was assessed by determining the %RSD values for 24 µg/ml, 30µg/ml, 36 µg/ml, (n=10) i.e at 80%, 100%, 120% level the resulting solutions were then reanalyzed by proposed method. The results are shown in table 2.

TABLE: 2 ACCURACY STUDIES

Level of accuracy	%RSD
80%	0.66
100%	0.59
120%	0.66

Precision

Precision of the methods was studied as intra-day, inter day and repeatability. Intra-day, study was performed by analyzing, the three different concentration of the drug (80%, 100%, 120%) in the same day. Inter-day precision was performed by analyzing three different concentration of the drug (80%, 100%, and 120%) for three days in a week [4]. Repeatability was performed by analyzing the three different concentration of drug (80%, 100%, and 120%) for 10 times at each concentration and the results are shown in table 3&4.

TABLE: 3 RESULTS FROM INTERDAY PRECISION

	INTERDAY PRECISION								
	DAY-1			DAY-2			DAY-3		
	24 µg/ml	30 µg/ml	36 µg/ml	24 µg/ml	30 µg/ml	36 µg/ml	24 µg/ml	30 µg/ml	36 µg/ml
%RSD	0.27	0.35	0.55	0.34	0.37	0.38	0.34	0.31	0.25

TABLE:4 RESULTS FROM INTRADAY PRECISION & REPEATABILITY

	INTRA DAY PRECISION						REPEATABILITY		
	10.30AM			3.30PM					
	concentration(µg/ml)			concentration(µg/ml)			Concentration(µg/ml)		
	24	30	36	24	30	36	24	30	36
% RSD	0.44	0.54	0.20	0.41	0.25	0.25	0.36	0.38	0.30

RUGGEDNESS OF TEST METHOD

ANALYST TO ANALYST

Analyst to Analyst variability study was conducted with different analysts under similar conditions at different concentration levels (24 µg/ml, 30 µg/ml, 36 µg/ml). Triplicate samples were prepared and each was analyzed as per test method and the results are shown in table 5.

TABLE 5: RESULTS FROM RUGGEDNESS

	RUGGEDNESS					
	Analyst-1			Analyst-2		
	24 µg/ml	30 µg/ml	36 µg/ml	24 µg/ml	30µg/ml	36 µg/ml
%RSD	0.56	0.66	0.16	0.28	0.35	0.28

RESULTS AND DISCUSSION

Development and validation of spectro photometric method for the estimation pioglitazone could be used as a valuable analytical tool in routine analysis, to check the batch to batch variations. After the drug is approved, pharmaceutical validation and development of finger printing are necessary to ensure that the drug product will meet/set pharmaceutical standards for identity, strength, quality, purity, safety and efficacy.

The wavelength 270nm (λmax for Pioglitazone) was selected for analysis of the drugs in 6N glacial acetic acid and linearity was observed in the range 10-50 µg/ml (r =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated with reference to linearity, accuracy, precision, and specificity. The accuracy of the methods was assessed by recovery studies at three different concentration levels. Molar absorptivity (e), low values of Sandell sensitivity indicated the high sensitivity of the proposed method.

The method was found to be precise as indicated by the repeatability, intra-day, inter-day analysis, showing %RSD less than 2. The results did not show any statistical difference between analysts suggesting that the method which is developed is rugged. The results of precision shown in table 3. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulation.

TABLE 4: ANALYSIS DATA OF TABLET FORMULATION

Drug	Label claim (mg/tab)	Assay(% of label claim) ± %RSD
Piosis	30	99.1±0.51

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