STUDY OF STRESSED DEGRADATION BEHAVIOR OF PIOGLITAZONE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION BY HPLC ASSAY METHOD

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A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of pioglitazone hydrochloride in pure and tablet forms. The method was validated with respect to linearity, precision, accuracy, and selectivity. The mean values of slope, intercept and correlation coefficent were 0.9998(r^2) respectively. The % COV values for repeatability and intermediate precision studies were < 2 indicates good precision of the method. The recovery of the drug ranged from 100.45-100.53**%** from a mixture of degradation products. The method was specific to drug and also selective to degradation products. The method showed a linear response for concentrations in the range of 10-65 µg/mL using 0.01 M potassium dihydrogen phosphate (pH 3.5) buffer: methanol [55:45] as the mobile phase with detection at 241.0 nm and a flow rate of 1.5 mL/min and retention time 6.15 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed; the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.

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Keywords: Pioglitazone hydrochloride, RP-HPLC, Degradation studies.

1. Introduction

Pioglitazone is a thiozolidinedione class of antidiabetic agents. It is selective agonists for nuclear peroxide proliferatoractived receptor-gamma. Pioglitazone HCl, Chemically [(±) -5-[[4-[2-(5-ethyl-2 Pyridinyl)ethoxy]phenyl]methyl]-2,4] thiazolidine-dione monohydrochloride, is thaizolidinedione derivative that highly selective agonist for peroxisome proliferator –activated receptor gamma (PPAR) and is used as an adjunct to diet to improve glycemic control in patient with type 2 diabetes (non-insulin dependent diabetes mellitus). The literature survey reveals the chromatographic methods are reported for simultaneous estimation of pioglitazone and its metabolites in human plasma, human serum, and urine [1-5]. It also reduces the insulin resistance in periphery and in the liver of patients. It increases glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation

of specific forms of the glucose transporter proteins. Pioglitazone is not official in any pharmacopoeia. Literature survey revealed only two HPLC methods for its determination in pharmaceuticals and two in human serum [8-10]. An Alkaline hydrolysis Stress stability study RP-HPLC method has also been reported for this combination [11].Our different approach in this combination Study of degradation behavior of Pioglitazone hydrochloride and development of a validated stability-indicating HPLC assay method.In recent times, there is an increase tendency towards the development of stability-indicating assay, using the approach of stress testing as mentioned in the ICH guidelines (Q1A). It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the

proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, light, oxidizing agent as well as susceptibility over a wide range of pH values [6, 7].

2. Experimental

2.1 Materials and methods

Chemicals

Pioglitazone HCl were obtained as gift samples from Dr. Reddys laboratories, Hyderabad. All the buffer materials and reagents used were of HPLC grade and purchased from Spectrochem, Mumbai, India. Nylon filter paper of 0.2µm (Ultipor) was purchased from Pall life science, Mumbai, India.

Instrumentation

Precision mentol heater (Biotech, Mumbai) with temperature regulator equippted with a reflux condencer were used for degradation study in acid, alkali and neutral conditions. Dry air oven was used to study the effect of dry heat. Photolytic study was carried out by exposing the drug to direct sunlight for 4h.The HPLC system equippted with an LC-10 AT *vp* solvent-delivery system with universal loop injector (Rheodyne 7725 i) of injection capacity of 20 µL. Detector consists of photodiode array detector SPD-10 AVP UV-Visible detector. Separation was carried out on a Phenomenex Luna C_{18} (5µm×25cm×4.6mm i.d) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC workstation. The work was carried out in an air-conditioned room maintained at temperature 25 ± 2^0C. Chromatograms were recorded using CLASS-VP software (Shimadzu, Kyoto, Japan).

Preparation of standard stock solution

The stock solution of Pioglitazone HCl (100 µg/mL) was prepared by dissolving 10. mg Pioglitazone HCl sample (99.98%) which is equivalent to 10.00 mg Pioglitazone HCl with mobile phase in a standard 100 mL volumetric flask. The stock solution of Pioglitazone HCl (100.0 µg/mL) was prepared by dissolving 10.01 mg of Pioglitazone HCl as internal standard (99.5%) with mobile phase in a standard 100 mL volumetric flask.

Forced degradation studies (stress testing)

The drug concentration for all stress studies was taken 1 mg/mL as per standard literature. The bulk drug was subjected to alkaline studies by adding 1.0 mL of the 1 N NaOH for 12 hours and neutralized with 1.0 mL of 1 N HCl acid. Similarly, the acidic studies was performed by adding 1.0 mL of 1 N HCl for 12 hours and neutralized with 1.0 mL of 1 N NaOH. Oxidation studies was performed on bulk drug by adding 1.0 mL of 5 % H_2O_2 for 12 hours and reduction studied was performed on bulk drug by adding 1.0 mL 1 % sodium borohydrate for 12 hours. Bulk drug was subjected to heat at 80 ºC for 12 hours. All samples were taken in different 10 mL volumetric flasks and dissolved in mobile phase. Final assay drug concentration and external internal standard was made up with mobile phase and injected in the chromatographic system. Susceptibility of the drug to dry heat was studied by exposing the solid drug to 60^0 C for 15 days in a hot air oven. Sampling was carried out every day to study its degradation behaviour. For all the stability study, the formation of degradable product was conformed by compairing the chromatogram of the degradable mixture with the blank solvent stored under normal condition and the drug solution kept under normal condition. Photolytic study was done by exposing the dry drug to direct sunlight for 12h. All stressed sample were analyzed by developed HPLC method [12-18].

Separation studies on stressed samples:

In all HPLC runs, the mobile phase was filtered through 0.2µm nylon membrane under vaccum and degassed before use. The injection volume was 20µl and the mobile phase flow rate was 1.5 ml min^{-1}, the analytical wavelength selected was 241 nm.HPLC studies were carried out on all reaction solution individually. Initially analyses were performed C_{18} column and mobile phase composed of potassium dihydrogen phosphate buffer: methanol (pH 3 adjusted with orthophosphoric acid). As the satisfactory resolution of the drug and the degradation products was not achieved, hence to get good resolution the method was further optimized by increasing the ratio of acetonitrile and it was found good resolution in the ratio of 55:45 (*v/v*) of potassium dihydrogen phosphate buffer: methanol.

3. Results and discussion

Analysis of stressed samples

The ICH stability guideline Q1A (R2) defines stress testing for new drug substances and drug products, to elucidate the intrinsic stability of the drug substances and drug products. The stress testing may also provide information about degradation pathways and selectivity of the applied analytical method. The drug was found to degrade extensively on reduction (Figure 2) and in acidic medium 1 N HCl. (Figure 3). Mild degradation was observed, when exposed to oxidation (Figure 1), basic (Figure 4) and thermal condition (Figure 5). Degradation % assay value given in Table 1.

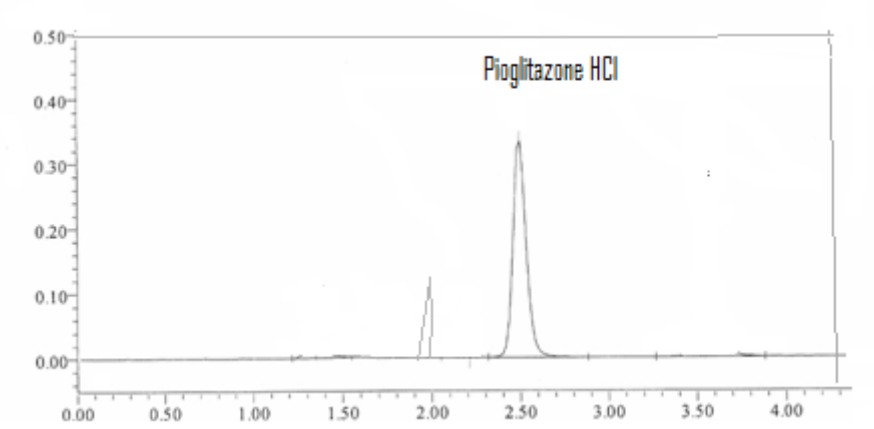

Fig. 1. HPLC Chromatogram representing degradation behaviour of Pioglitazone HCI in oxidation.

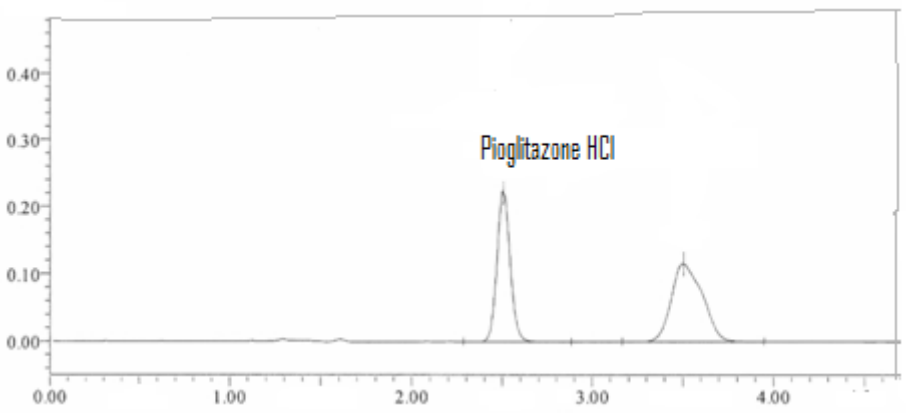

Fig. 2. HPLC Chromatogram representing degradation behavior of Pioglitazone HCI in reduction

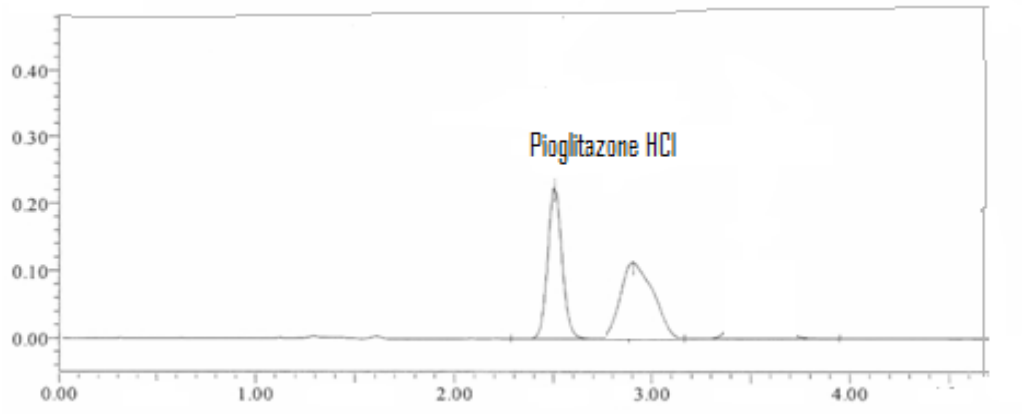

Fig. 3. HPLC Chromatogram representing degradation behavior of Pioglitazone HCIin acid

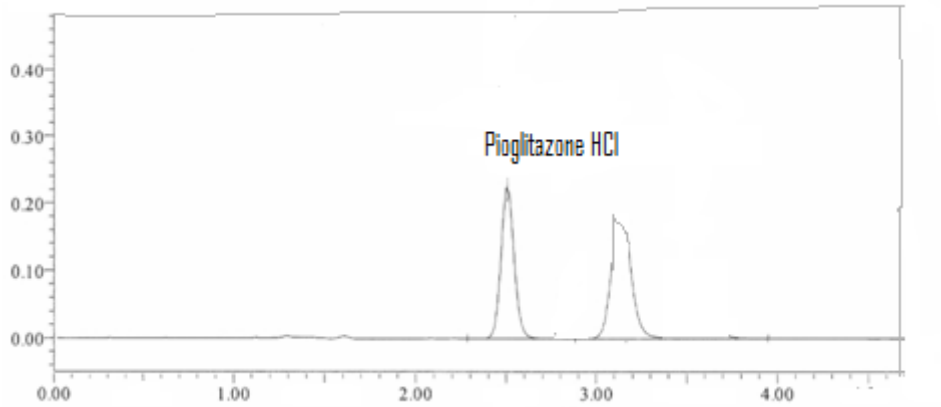

Fig. 4. HPLC Chromatogram representing degradation behavior of Pioglitazone HCIin base

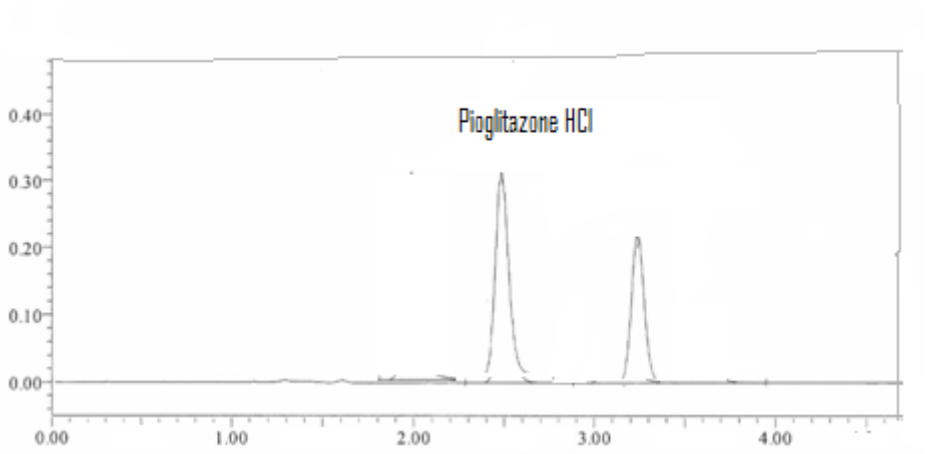

Fig. 5. HPLC Chromatogram representing thermal degradation behavior of Pioglitazone HCI.

Table-1 Summary of degradation products of Pioglitazone HCl under different stress conditions.

Sample exposure condition	% assay of drug after exposed to stress condition	% assay (impurity observed+% assay after exposed	% of degradation impurity observed
Acid 12h	88.15	12.65	94.54
Base 12h	90.21	10.95	98.25
Oxidation 12h	93.67	7.35	100.1
Thermal, 12h	95.12	5.85	99.41
Reduction 12h	96.32	4.54	98.16

Linearity

Several aliquots of standard stock solution (1.0, 2.5, 3.5, 4.0, 5.5, and 6.5 mL; 1 mL= 100µg mL-1) of Pioglitazone HCl drug were taken in different six standard 10.0 mL volumetric flasks and diluted up to mark with mobile phase. Evaluation was performed with UV detector at 241 nm. The peak area was recorded for all the peaks and calibration graph was obtained by plotting peak area ratio *versus* concentration of Pioglitazone HCl. The plot of peak area ratio against concentration of Pioglitazone HCl was found to be linear in the range of 10 to 65 µg/ mL with correlation coefficient of 0.9989.The respective slope, intercept and correlation coefficient given in Table 2.

Table 2. The calibration graph peak area ratio, slope, intercept and correlation coefficient.

Concentration in µg /mL	**Area**	**Area ratio**
10.00	438515	0.438
25.12	624185	0.624
35.29	794327	0.843
40.00	964174	0.964
55.51	1046294	1.04
65.11	1262179	1.26
Correlation coefficient		0.9986
Slope		0.0241
Intercept		0.3682

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined from standard deviation and slope method as per ICH guideline, for Pioglitazone HCl LOD was found to be 1.693 µg/ mL and LOQ was found to be 5.316 µg/ mL. The respective limit of detection, limit of quantitation and LOQ level precision is given in Table 3.

Table 3. Results of LOD and LOQ.

Concentration in µg /mL	Area
10.00	438515
25.12	624185
35.29	794327
40.00	964174
55.51	1046294
65.11	1262179
Correlation coefficient	0.9992
Slope	31594.174
S.D	43975.841
LOD	1.693
LOQ	5.316

Table 4. Results of the study accuracy.

Spiked conc., ppm	Level %	Recovered conc., ppm	% Recovery	% RSD	Mean	S.D.
15.25	80	15.42	101.21	0.42	101.11	0.39
		15.29	100.03			
		15.36	101.01			
25.80	100	25.89	100.04	0.63	101.28	0.72
		26.66	101.23			
		25.98	100.11			
32.64	120	31.98	99.97	0.76	100.31	0.83
		32.88	100.02			
		33.13	101.11			

Accuracy

To study accuracy of the method, recovery experiment was carried out by spiked concentrations. A known quantity of drug substance corresponding to 80, 100 and 120% was spiked, to determine accuracy (recovery) against 100% working standard. The accuracy was expressed as the percentage of analytes recovered by the assay. The results indicate the method is highly accurate for determination of the Pioglitazone HCl. Table 4 lists the recoveries of the drugs from a series of spiked concentrations.

Precision

Repeatability was studied by carrying out system precision and method precision. System precision was determined from results for six replicate injections of the system suitability standard solutions. The relative standard deviations were less than 2%. Method precision was determined by estimated the percentage assay of Pioglitazone HCl. The relative standard deviation was 0.76 Table 5.

Table 5. Results of the study of relative standard deviation.

S.No	%Assay
1	99.78
2	100.03
3	100.11
4	101.13
5	99.98
6	100.01
Mean	100.21
S.D	0.51
%RSD	0.76

Table 6. Theoretical plates, resolution, tailing factor

Replicate n=6	Area ratio	Tailing factor	Resolution
Mean	3.8416	1.932	5.82
%RSD	0.76	----------	----------

Degradation studies of Pioglitazone HCl-

HPLC studies of sample obtained on stress testing of Pioglitazone HCl under different conditions using potassium dihydrogen phosphate buffer: methanol (55:45) as the mobile solvent system suggested the following degradation studies. It was observed that the drug gets slowly degraded in strongly acidic conditions over a period of time. On reflux in 1 N HCl (6h) and further for 12h there is no degradation. The degradation of the drug resulted in the rise of one extra peak at 3.253 in 1.0 N HCl (12h). This indicates that the drug is hydrolysed under acid conditions, to a chromatographic compound.In alkali, the drug was found to decompose almost 60-70% and then 70-80% after refluxing for 2h and then continuing 3h in 1 N NaOH respectively. As shown in chromatogram degradation of the drug resulted in the rise of one extra peak at 3.121 min.In neutral condition, mild degradation only 5-7 % was seen after reflux for 24h at 80^0C with a decrease in peak height. The drug was found to be stable in 5% H_2O_2 for 6h at room temperature. However about 5-10 % drug degradation was observed on exposure to 10% H_2O_2 for 24h. One small degradation product peaks at 2.841 min was seen and there was significant rise in the height of the peak with time. This signifies that the drug was degraded in oxidative conditions to chromatographic compounds. Photolytic study was carried out in dry form. Here the drug was directly exposed to the sunlight for 4h on a hot sunny day. Pioglitazone HCl was found to be degraded in very negligible amount with a reduction in peak height. Only (3%) of the drug was found to be degraded in to non-chromatographic product. It shows Pioglitazone HCl was almost stable in the photolytic studies.Pioglitazone HCl was found to be degraded in negligible amount as two new peaks of degradation products were appear at 3.872 min. Only (3-8%) of the drug was found to be degraded hence Pioglitazone HCl was almost stable after exposing the drug to 80^0 C for 20 days.

Development of stability-indicating method

It was observed that satisfactory resolution of Pioglitazone HCl and its degradation products formed under various conditions was achieved when the analyses was performed by using a mixture of potassium dihydrogen phosphate buffer and methanol (pH 3 adjusted with

orthophsophoric acid) in the ratio of 55:45 (*v/v*) as mobile phase at a flow rate of 1.5 ml min^{-1}. UV detection was performed at 241nm.

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