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Method Development and Validation of Forced Degradation Studies of Pioglitazone Hydrochloride by Using UV Spectroscopy

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ABSTRACT

Pioglitazone hydrochloride is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus and also known as non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes. A simple, an accurate and economic, precise and reproducible UV Spectroscopy method has been developed for the estimation of Pioglitazone hydrochloride tablet dosage form and validated by ICH guidelines. The standard (10 µg/ml) was scanned between 200-400 nm and maximum absorption was recorded at 270 nm. The assay results are found to be 99.7±0.352. The linearity range of 10-50 µg/ml proved that it obeyed Beer's Law and the correlation coefficient (r^2) was found to be 0.99986 at 270 nm with an intercept of 0.008and a slope of 0.011 with RSD 0.187 complied ICH. The pH degradation studies of tablet formulation were found to be less at pH 6-8. The force degradation studies of pioglitazone tablet formulation were done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 13.07% for 60min, 17.95% for 90min. Stress degradation by hydrolysis under acidic condition by using 3N HCl and product degradation was found to be 19.75% for 60min and 21.79% for 90 min. Dry heat-induced degradation was done by using 700c temperature was found to be 0.14% for 48 hrs. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 12.65%for 15 min. Photolytic degradation was found to be 12.53% for 3hrs and 18.36% for 6hrs.

INTRODUCTION

Pioglitazone is an oral antidiabetic agent belonging to the class of thiazolidinedione that acts primarily by decreasing insulin resistance. It is used in the management of type 2 diabetes mellitus. It improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis also improves glycemic control while reducing circulating insulin levels. Pioglitazone [(±)-5-[[4-[2-(5-ethyl-2- pyridinyl) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione monohydrochloride belongs to a different chemical class and has a different pharmacological action than the sulfonylureas, metformin, or the -glycosidase inhibitors [(1)]. The chemical structure for Pioglitazone Hydrochloride was shown in Figure no.1.

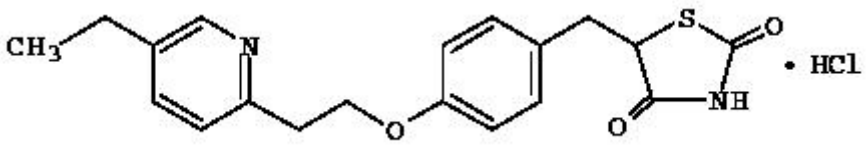

Figure no. 1: Chemical structure of Pioglitazone HCl

Determination of pioglitazone by various analytical methods like Spectrophotometric method[(2)] and HPLC and MECK method[(3)] in tablet dosage form, HPLC and solid phase extraction method in human serum[(4)] and in dog serum[(5)], HPLC and LC-MS in human plasma[(6), (7)]have been reported. Pioglitazone is not official in any pharmacopeia. There is a need for a simple, rapid, cost-effective and reproducible method for assay of pioglitazone in its dosage forms But there was no reported method for the Forced degradation studies of pioglitazone hydrochloride by using UV spectroscopy. So the present work is to carry out the force degradation studies along with its pH degradation studies. The method was validated according to the ICH (Q2A1995) guidelines [(8)] Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behavior can be used to improve a drug product. The available regulatory guidance provides useful definitions and general comments about degradation studies [(9)] The International Conference on Harmonization (ICH) guidelines [(10-11)]Indicates that stress testing is designed to determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used. ICH guidelines stability testing of new drug substances and products' Q1A (R2) [(10)] and (Q1B) [(11)] requires that stress testing should be carried out to elucidate the

substance. It suggests that the degradation products that are formed under the variety of condition should include the effect of temperature, appropriate oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH value. In the guideline, the study of the effect of temperature is suggested to be done in 100C increment above the accelerated temperature (500C, 600C, etc.) and that of humidity at a level of 75 % or greater. Exact details are however provided for the study of oxidation, photolysis, and hydrolysis at different values [12]

MATERIALS AND METHODS

Instrument

Absorption spectral measurements were carried out with a UV – Visible spectrophotometer (Shimadzu Model 1700) using UV Probe software version 2 was employed with a spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells.

Chemicals

Water used for dilution was distilled in the laboratory. A double beam UV spectrophotometer (Shimadzu UV-1800) was used with 1 cm matched quartz cell. Tablet formulation [Pioz 15, USV Ltd., Baddi, Solan- Dist, Himachal pradesh] were procured from a local pharmacy with a labeled amount of 15 mg per tablet.

Preliminary solubility study of drug

The solubility of the drug was determined at 28±1 C.A small quantity of standard drugs were dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, dimethyl sulfoxide, dimethylformamide, 0.1 N HCl, chloroform, acetonitrile, and pH 4, 7, 9.2 buffer solutions. The results are reported in table no. 1.

Solvent Selection:

Various solvents were selected for the solubility studies and it was found that Pioglitazone was soluble in the following solvents; dimethyl sulfoxide, dimethylformamide, methanol, 0.1 N HCl, chloroform, acetonitrile, *etc.* In the present investigation, methanol was selected as a solvent.

Selection of analytical wavelength and absorption maxima:

Appropriate 10µg/ml dilutions were prepared for the drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained was derivatized for zero order spectroscopy. This zero order spectrum was selected for the analysis of the drugs. The absorption maximum was found at 270 nm which can be further used for analysis as shown in Figure no.2.

Preparation of stock solutions:

Standard Pioglitazone 100mg was weighed and transformed to a 100 ml volumetric flask and dissolved in 25 ml of methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml (Stock solution A). From this stock solution A, pipette out 5 ml and place into 50 ml volumetric flask. The volume was made up to the mark with methanol to give a solution containing 100 µg/ml (Stock solution B).

Selection of analytical concentration range:

From the standard stock solution B of Pioglitazone, appropriate aliquots 1, 2, 3, 4 and 5 ml were pipetted out in 10 ml volumetric flasks and dilutions were made with methanol to obtain working standard solutions of concentrations from 1-50µg/ml. Absorbance for these solutions was measured at 270 nm. For standard solution, the analytical concentration range was found to be 1-50 µg/ml and overlain spectra were obtained and optical characteristic and linearity data was reported in table no.2.

Calibration curve for the Pioglitazone:

Appropriate volumes of aliquots from standard Pioglitazone stock solution B were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 10, 20, 30, 40 and 50µg/ml. The absorbance value of each solution against methanol as a blank was measured at 270nm. From that absorbance value, regression equation and correlation coefficient (r^2) are determined and presented Figure no.3.

Analysis of Pioglitazone from Tablet Dosage form:

Twenty tablets of formulation were weighed and finely powdered. The powder equivalent to 100mg of Pioglitazone was accurately weighed. It was then transferred to a volumetric flask of 100 ml capacity containing 25 ml of methanol and sonicated for 30 min. The flask was shaken and the solution was filtered through Whatmann filter paper (No. 41) into 100 ml volumetric flask Volume was made up to the mark with methanol to give a solution of 1000 µg/ml (Stock solution A). From this solution, 5 ml was taken and placed in 50 ml volumetric flask. The volume was made up to the mark using methanol to give a solution of 100 µg/ml (Stock solution B). From the stock solution B, 2.0 ml was taken and diluted to 10 ml to give 20 µg/ml and it was further used for the estimation of Pioglitazone. The result was reported in Table no. 3.

METHOD VALIDATION

Validation parameters:

The method was validated with reference to accuracy, precision, and ruggedness.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding a known amount of standard solution of the drug to pre-analyzed tablet solutions. The resulting solutions were then reanalyzed by proposed methods; the results are reported in Table no.4.

Precision

The precision of the methods was studied as intra-day, interday and repeatability. The intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing the same concentration of drugs for six times. The results are reported in Table no.5.

Ruggedness

The ruggedness of the proposed method is determined by analysis of aliquots from the homogenous slot by different analysts using similar operational and environmental conditions. The results are reported in Table no. 6.

pH DEGRADATION STUDIES:

The pH effect on the drug was carried out by using 0.1N Hydrochloric acid, 2N Hydrochloric acid, 0.1N Sodium Hydroxide, and 2N Sodium Hydroxide solution. The drug solutions (20µg/ml) from pH 0-14 were prepared in the manner as shown in table 7 and these were allowed to stands for 4 hours. Finally, the absorbances were measured at 270nm. The K value for 1st order kinetics was determined by using the formula:

$$K= (2.303/t) \log (Co/C)$$

Where K= 1st order rate constant,

Co. =initial drug concentration,

C=final drug concentration

The results were reported in table no.8.

Degradation Studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Pioglitazone hydrochloride using the method developed.

Stress degradation by hydrolysis under the acidic condition:

To 3 ml of stock solution(1000µg/ml) of Pioglitazone, 1 ml of 3 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. Then, the volumetric flask was kept at the normal condition for 90 minutes. After 60 min. the time interval, 1 ml of solution was pipetted out from this flask, neutralized and diluted with methanol in order to make the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (30µg/ml). This solution was taken in the cuvette. For the

blank, 0.5 ml solution of 3N HCl and 0.5 ml solution of 3N NaOH were diluted with methanol in 10 ml of volumetric flask. After 90 minutes, again 1ml of the solution was pipetted out from the flask and the above procedure was repeated.

Stress degradation by hydrolysis under the alkaline condition:

To 3 ml of stock solution of Pioglitazone 1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and made up the volume to the mark with methanol. The volumetric flask was kept at the normal condition for 90 min. After 60 min time interval, 1ml of the solution was pipetted out from this flask, neutralized and diluted with methanol in order to make the volume up to 10 ml and the dilutions were carried out to achieve the appropriate concentration (20µg/ml). The solution was then taken in the cuvette. For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml solution of 0.1N NaOH diluted with methanol in 10 ml of volumetric flask. After 90 minutes 1ml of the solution was again pipetted out from the flask and the above procedure was repeated.

Dry heat-induced degradation:

Pioglitazone sample was taken in a Petri plate and exposed to a temperature of 70°c for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with methanol in order to make the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml) and the solution was taken in the cuvette for the UV-VIS Analysis.

Oxidative degradation:

To 1.5 ml of the stock solution of Pioglitazone (1000µg/ml), 1 ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. The volumetric flask was then kept at room temperature for 15 min. For the blank, 1 ml of the 30 % w/v of hydrogen peroxide was kept at the normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on a boiling water bath to remove the excess of hydrogen peroxide. Finally, after 15 minutes dilutions were made from the stock solution to achieve the required concentration (30µg/ml). The solution was then taken in a cuvette and analyzed in UV.

Photolytic degradation:

Sample of Pioglitazone was exposed to a near ultraviolet lamp in photostability chamber providing illumination of not less than 1.2 million lux hours. Ten milligrams sample was dissolved in methanol and volume made up to 10 ml. From this solution, appropriate dilution (30µg/ml) was made using methanol and taken in the cuvette for the U.V analysis.

The results were reported in table no 9.

RESULTS AND DISCUSSION

Pioglitazone was freely soluble in Methanol and Dimethyl sulfoxide. Methanol was chosen as a solvent. The drug has maximum absorbance at 270nm. The optical characteristic of the drug was found to be Beer's law limits 1-50 µg/ml, the Correlation coefficient is 0.99986, Std error is 0.003302, Molar Absorbance is 4620.504. The drug sample was analyzed by UV spectroscopy using methanol as solvent and the average content of drug present in the formulation was found to be 100.6%. The RSD of accuracy studies was found to be 99.7±0.352. The %RSD of precision was found to be 0.0209 to 0975.The% recovery of ruggedness was found to be 99.31±0.1636 and 99.67±0.5953. The pH degradation studies of tablet formulation were found to be less at pH 6-8. The force degradation studies of pioglitazone tablet formulation were done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 13.07% for 60min, 17.95% for 90min. Stress degradation by hydrolysis under acidic condition by using 3N HCl and product degradation was found to be 19.75% for 60min and 21.79% for 90 min. Dry heat-induced degradation was done by using 700c temperature was found to be 0.14% for 48 hrs. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 12.65%for 15 min. Photolytic degradation was found to be 12.53% for 3hrs and 18.36% for 6hrs.

Table no.1: Solubility data for pioglitazone

S. No.	Solvent	Solubility status
1.	Distilled water (HPLC and spectroscopic grade)	Insoluble
2.	Ethanol	Slightly soluble
3	Methanol	Freely soluble
4.	Acetonitrile	Soluble
5.	Dimethyl sulfoxide	Freely soluble
6.	Dimethylformamide	Soluble
7.	Chloroform	Soluble
8.	Isopropyl alcohol	Insoluble
9.	0.1NaoH	Soluble
10.	Buffer P^H 4 solution	Insoluble
11.	Buffer P^H 7 solution	Insoluble

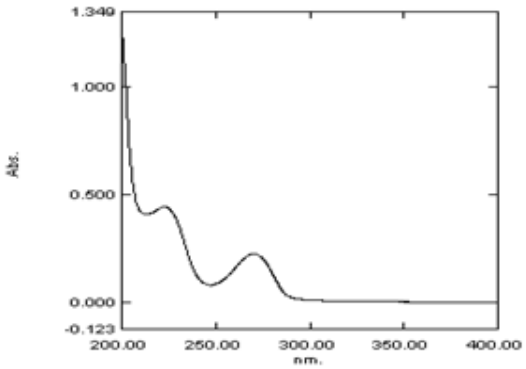

Figure no 2 : UV Spectra of Pioglitazone Standard

Table no.2: UV Optical characteristic and linearity data

Parameters	Pioglitazone
λ max (nm)	270
Beer's law limits in µg/ml	1-50
Correlation coefficient	0.99986
Regression equation Y=mx+c	Y=0.011x+0.008
Intercept(c)	0.008
Slope	0.011
Std error	0.003302
Molar Abs	4620.504
Sandel's	0.091324

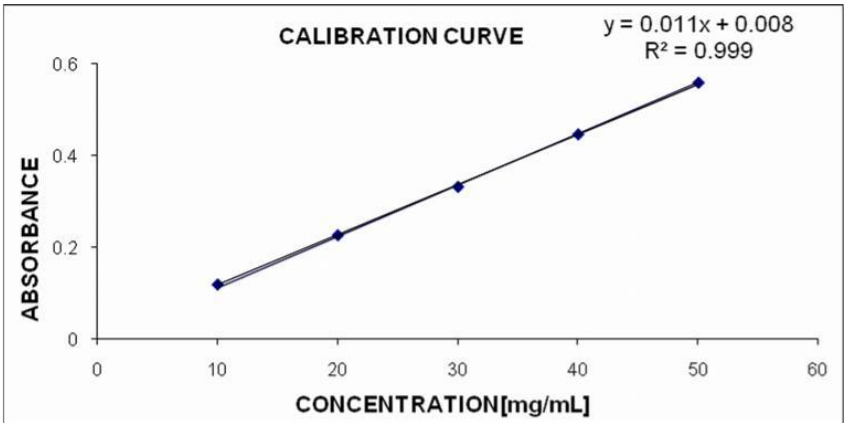

Figure no 3: UV Linearity graph of Pioglitazone

Table no 3: Analysis data of Tablet formulation by UV

Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D.*	% COV	S.E*
PIO	15	15.1	100.6	0.115	0.066	0.005

PIO: Pioglitazone, S.D: Standard deviation, COV: Coefficient of variation,

S.E: Standard error

Table no. 4: Results of Accuracy studies by UV spectroscopy

Level of recovery	Amount of sample (µg/ml)	Amount of drug added (µg/ml)**	Amount of drug recovered (µg/ml)**	% Recovery ± S.D**
80%	10	8.0	7.98	99.7±0.352
100%	10	10.1	9.92	99.2±0.207
120%	10	12.0	11.97	99.7±0.215

** is an average of six determinations

Table no. 5: Precision study data of Pioglitazone by UV spectroscopy

Concentration (µg/ml)	Inter-day Absorbance mean ± SD**	% RSD	Intra-day Absorbance mean ± SD**	% RSD
10	0.120±0.00041	0.342	0.120±0.000117	0.975
20	0.227±0.00052	0.224	0.227±0.00052	0.225
30	0.332±0.00063	0.192	0.332±0.00041	0.124
40	0.446±0.00041	0.089	0.446±0.00052	0.113
50	0.558±0.00052	0.092	0.558±0.00117	0.209

** is an average of six determinations

Table no. 6: Ruggedness study data of Pioglitazone by UV

Sample	Label claim(mg)	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovery ±SD**	Amount found (mg)	% Recovery ±SD**
Pioz	15	14.89	99.31 ±0.1636	14.95	99.67 ±0.5953

** is an average of six determinations

Table no. 7: Preparation of sample solution of pH 0-14 for pH stability

pH	Amount of Drug solution added (100 µg/ml) in ml	Amount of 0.1N NaOH solution added(ml)	Amount of HCl added in ml
0	2.5	2.5	12.5 of 2N HCl
1	2.5	2.5	1.25 of 2N HCl
2	2.5	2.5	1.25 of 0.2N HCl
3	2.5	2.5	1.25 of 0.02N HCl
4	2.5	2.5	1.25 of 0.002N HCl
5	2.5	2.5	1.25 of 0.002N HCl
6	2.5	2.5	1.25 of 0.002N HCl
7	2.5	2.5	
8	2.5	2.5	12.5 of 2N NaOH
9	2.5	2.5	1.25 of 2N NaOH
10	2.5	2.5	1.25 of 0.2N NaOH
11	2.5	2.5	1.25 of 0.02N NaOH
12	2.5	2.5	1.25 of 0.002N NaOH
13	2.5	2.5	1.25 of 0.0002N NaOH
14	2.5	2.5	1.25 of 0.00002N NaOH

Table 8: pH Degradation Results:

pH	Absorbance (at 270 nm)	Concentration (µg/ml)	% Drug degraded	k Value	Log k
0	0.45	17.1103	14.45	0.039	-1.4089
1	0.452	17.1863	14.07	0.0379	-1.4214
2	0.449	17.0722	14.64	0.0396	-1.4023
3	0.458	17.4144	12.93	0.0346	-1.4609
4	0.458	17.4144	12.93	0.0346	-1.4609
5	0.502	19.0875	4.56	0.0117	-1.9318
6	0.521	19.8099	0.95	0.0024	-2.6198
7	0.515	19.5817	2.09	0.0053	-2.2757
8	0.508	19.3156	3.42	0.0087	-2.0605
9	0.499	17.0722	14.64	0.0396	-1.4203
10	0.422	16.0456	19.77	0.0551	-1.2588
11	0.392	14.9049	25.48	0.0735	-1.1337
12	0.378	14.3726	28.14	0.0826	-1.083
13	0.342	13.0038	34.98	0.1076	-0.9682
14	0.331	12.5856	37.07	0.1158	-0.9363

Table no. 9: Results of Stress Degradation Studies

Condition	Time	% of Degradation
0.1N NaOH(1ml)	60min	13.07%
	90min	17.95%
3N HCl(1ml)	60min	19.75%
	90min	21.79%
30% Hydrogen Peroxide(1ml)	15min	12.65%
Dry Heat 70°	48hr	0.14%
Photolytic	3hr	12.53%
	6hr	18.36%

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