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# A Stability indicating First Order Derivative Spectroscopic Method development and Validation for Estimation of Pioglitazone

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**Abstract:** Pioglitazone hydrochloride is an oral anti-diabetic 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 231.5 nm.The assay results were found to be 100.52%. The linearity range of 15-65 µg/ml proved that it obeyed Beer's Law and the correlation coefficient (r2) was found to be 0.9983 at 270 nm with an intercept of 0.0008and a slope of 0.0018 with RSD less than 2% complied ICH. The pH degradation study of API was found to be less at pH 7-12. The force degradation studies of Pioglitazone was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 5.76% for 60min, 9.61% for 90min. Stress degradation by hydrolysis under acidic condition by using 3N HCl and product degradation was found to be11.53% for 60min and 21.15% for 90 min for API. Dry heat induced degradation was done by using 70ºC temperature was found to be 1.93 % for API for 48 hrs. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 19.23% at 15 min. Photolytic degradation was found to be 9.61 % for 3hrs and 15.38% for 5 hrs for API.
**Keywords:** Pioglitazone Hydrochloride, 1st Order Derivative Spectroscopy, Stability Indicating method, Forced Degradation Studies.

## INTRODUCTION

Pioglitazone is an oral anti-diabetic drug of class thiazolidinediones that acts primarily by declining insulin resistance. It is used in the treatment 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-] thiazolidine dione monohydrochloride[1]. Determination of Pioglitazone by various analytical methods like Spectrophoto metric method[2,3] and HPLC and MECK method[4] in tablet dosage form, HPLC and solid phase extraction method in human serum[5] and in dog serum[6], HPLC and LC MS in human plasma[7] have been reported. But these methods are sophisticated, expensive and time consuming when compared to simple UV spectrophoto metric method.

Pioglitazone is not official in any pharmacopoeia. There is a need for a simple, rapid, cost effective and reproducible method for assay of Pioglitazone in its dosage forms. Therefore, it was notion of interest to

develop simple, speedy, accurate and cost effective method for the analysis of Pioglitazone in its tablet formulation. This paper describes a stability indicating method development and validation of simple, specific, sensitive, accurate and precise 1st order derivative UV Spectrophotometric method[8,9,10] for the estimation of Pioglitazone in bulk and its formulation. Forced degradation studies of Pioglitazone by 1st order derivative spectroscopy have yet not been reported. Consequently, the present work is to carry out forced degradation studies of Pioglitazone after its first order method development and validation by 1st order derivative spectroscopy.

Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. 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 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.

In the guideline, the study of effect of temperature is suggested to be done in 100 ºC increment above the accelerated temperature (500ºC, 600 ºC 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 pH values [12, 13, 14]

## MATERIALS AND METHODS:

**Instrument:** Double beam UV–Visible spectrophotometer (Shimadzu Model 1800) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.1 nm using a pair of 5 cm matched quartz cells.

**Chemicals:** 0.1N NaOH used as solvent was prepared using triple distilled water. Pioglitazone was kindly supplied by Aurobindo Pharmaceuticals, Aurangabad. Pioglitazone [Pionorm and Pioz (Pioz 15, USV Ltd., Baddi, Solan- Dist, Himachal pradesh)] were obtained from local pharmacies. NaOH from Qualigens Fine Chemicals, Mumbai.

**Solubility Profile of Drug:** 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, 0.1 N HCl, chloroform, acetonitrile and pH 4, 7, 9.2 buffer solutions. The results are reported in table 1.

**Table 1: Solubility Profile**

| S. No. | Solvent | Observation |
|---|---|---|
| 1. | Chloroform | Soluble |
| 2. | Methanol | Soluble |
| 3. | Ethanol | Slightly Soluble |
| 4. | 0.1 N NaOH | Soluble |
| 5. | 1 N NaOH | Soluble |
| 6. | 2 N NaOH | Soluble |
| 7. | Distilled water | Insoluble |
| 8. | 0.1 N HCl | Soluble |
| 9. | 1 N HCl | Soluble |
| 10. | 2 N HCl | Soluble |
| 11. | Buffer pH-4 | Soluble |
| 12. | Buffer pH-7 | Soluble |
| 13. | Buffer pH-9.2 | Soluble |
| 14. | Acetone | Very Slightly Soluble |
| 15. | Acetonitrile | Very Slightly Soluble |

It was found that Pioglitazone was soluble in the following solvents; 0.1N 1N 2N NaOH, methanol, 0.1N 1N 2N HCl, chloroform, Buffer pH 4 7 9.2, *etc.* In the present analysis 0.1N NaOH was selected as a solvent.

**Selection of analytical wavelength:** 10µg/ml dilution was prepared for drug from the standard stock solution accurately and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained was transformed for first order spectroscopy. This first order spectrum was selected for the analysis of the drugs. The detection wavelength selected was 231.5nm because the drug showed sufficient absorption and lower interference and low quantity of the drug may be detected correctly which can be further used for analysis as shown in Fig.1

**Preparation of Stock and Working Stock Solution:** Standard Pioglitazone 25 mg was weighed accurately and transferred to a 25 ml volumetric flask and dissolved in 20 ml of 0.1N NaOH. The flask was shaken and volume was made up to the mark with 0.1N NaOH to give a solution containing 1000 µg/ml (Stock solution).

From this stock solution, 10ml was pippetted out and placed into 100 ml volumetric flask. The volume was made up to the mark with 0.1N NaOH to give a solution containing100µg/ml (Working Stock solution).

**Determination of Linearity and Concentration Range**: From the working stock solution of Pioglitazone, appropriate aliquots 1.5, 1.8, 2.1, 2.4, 2.7 and 3 ml were pipetted out in 10 ml volumetric flasks and dilutions were made with 0.1N NaOH of concentrations from 1.5-3.0µg/ml. D1 value for these solutions were calculated at 237.5 nm. For standard solution analytical concentration range was found to be 1.5-3.0 µg/ml and overlain spectra was obtained and optical characteristic and linearity data was reported in Table 2.

From that D1 value, regression equation and correlation coefficient ($R^2$) are determined and reported Fig.2.

**Fig 1: 1st Order Derivative spectra of Pioglitazone**

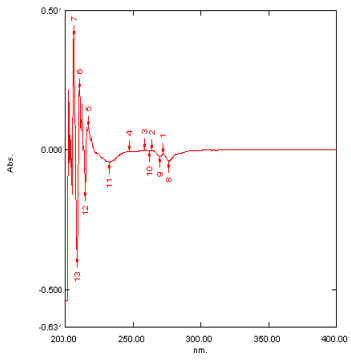


**Fig 2: Calibration Curve of Standard Pioglitazone**

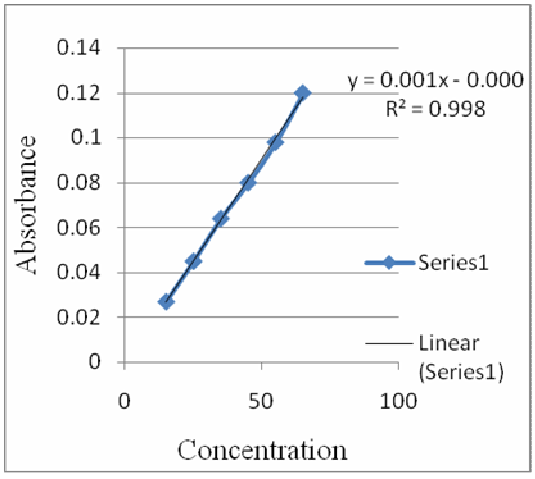

**Table 2: Validation data**

| Validation criteria | Result |
|---|---|
| Absorbance Maxima | 231.5nm |
| Linearity response | 15-65µg/mL |
| Beer' law limit | 15-65µg/mL |
| Slope | 0.0018 |
| Intercept | 0.0008 |
| Regression equation | y=0.0018x– 0.0008 |

## METHOD VALIDATION

**Validation Parameters:** The method was validated with reference to accuracy, precision, robustness and recovery studies were performed.

**Precision:** The drug was subjected to system precision, method precision, Intra and Inter-Day precision. Intra-day study was performed by analyzing, any one concentration of drug for different time interval in the same day. Inter-day precision was performed by analyzing any one concentration of the drug for consecutive days in a week at same time. The results are reported in Table 3.

**Robustness:** Robustness of the proposed method is determined by analysis of six aliquots from homogenous slot by different analysts, different temperature and different pipette using similar operational and environmental conditions. The results are reported in Table 3.

**Sensitivity:** Sensitivity is the capacity of the test procedure to record small variation in concentration. For spectrophotometry sensitivity is measured in terms of Sandell's Sensitivity (л). Six different sets of dilution were prepared in the range and average D1 was calculated. The results are reported in Table 3.

**Table 3: Results of Different Parameter of Analytical Method**

| Validation criteria | Result |
|---|---|
| System precision | %RSD< 2% |
| Robustness | %RSD< 2% |
| Method precision | %RSD< 2% |
| Intraday precision | Up to 5 hrs |
| Interday precision | Up to 3 days |
| Sensitivity | 55.672µg/cm$^2$/0.001 AU |

**Recovery Studies:** The accuracy of a method is expressed as the closeness of agreement between the found value and reference value. The accuracy of the proposed method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The recovery studies were carried out by adding known amount of standard solution of the drug to preanalysed tablet solutions. The resulting solutions were then reanalyzed by proposed methods; the results are reported in Table 4.

**Table 4: Results of Recovery Studies by 1$^{st}$ Order Derivative Spectroscopy**

| Level of recovery | Amt of sample | Amt obtained | % Recovery |
|---|---|---|---|
| 50% | 12 | 19.25 | 110.41 |
| 100% | 12 | 25.37 | 111.41 |
| 150% | 12 | 30.36 | 103.05 |

**Analysis of Pioglitazone from Tablet Dosage form / Assay:**
Twenty tablets of formulation were weighed and finely powdered. The powder equivalent to 100 mg of Pioglitazone was accurately weighed and approximately 50-60 mL of 0.1N NaOH was added and stirred until it gets dissolved and sonicated for 5-10 mins. The volume of solution was made upto 100 mL. The solution was filtered. Then 10 mL of filtrate was diluted up to 100 ml with 0.1 N NaOH. 2.1 mL of resulting solution was diluted up to 10 mL. D1 value of the final solution was recorded at 231.5 nm. The result is reported in Table 5.

**Table 5: Assay Results**

| Drug | Label claim mg/tab | Amt found mg/tab | % Assay |
|---|---|---|---|
| Pionorm | 15 | 15.079 | 100.52 |

**FORCED DEGRADATION STUDIES OF PIOGLITAZONE**

**1. 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 and these were allowed to stands for 4 hours. Finally the absorbance was measured at 231.5 nm. 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 6 and Table 7.

**Table 6: Preparation of sample solution of pH 0-14 for pH stability**

| S.No. | pH | Amount of HCl / NaOH added in mL |
|---|---|---|
| 1 | 0 | 0.95 mL 0.1 N HCl |
| 2 | 1 | 0.9 mL 0.1 N HCl |
| 3 | 2 | 0.85 mL 0.1 N HCl |
| 4 | 3 | 0.8 mL 0.1 N HCl |
| 5 | 4 | 0.7 mL 0.1 N HCl |
| 6 | 5 | 0.65 mL 0.1 N HCl |
| 7 | 6 | 0.6 mL 0.1 N HCl |
| 8 | 7 | 0.55 mL 0.1 N HCl |
| 9 | 8 | 0.55 mL 0.1 N HCl |
| 10 | 9 | 0.4 mL 0.1 N HCl |
| 11 | 10 | 0.2 mL 0.1 N HCl |
| 12 | 11 | 0.2 mL 0.1 N HCl |
| 13 | 12 | 0.1 mL 0.1 N HCl |
| **15** | 13 | 0.2 mL 0.1 N NaOH |
| **16** | 14 | 2.5 mL 1 N NaOH |

**Table 7: pH Degradation Results**

| pH | D1 Value | Conc (µg/mL) | % Drug degraded | K Value | Log K |
|---|---|---|---|---|---|
| **0** | 0.029 | 15.263 | 23.865 | 0.0771 | -1.113 |
| **1** | 0.029 | 15.263 | 23.865 | 0.0771 | -1.113 |
| **2** | 0.029 | 15.263 | 23.865 | 0.0771 | -1.113 |
| **3** | 0.031 | 16.315 | 18.425 | 0.0602 | -1.219 |
| **4** | 0.032 | 16.842 | 15.790 | 0.0489 | -1.310 |
| **5** | 0.035 | 18.421 | 7.895 | 0.0233 | -1.632 |
| **6** | 0.035 | 18.421 | 7.895 | 0.0233 | -1.632 |
| **7** | 0.036 | 18.947 | 5.265 | 0.0152 | -1.818 |
| **8** | 0.036 | 18.947 | 5.265 | 0.0152 | -1.818 |
| **9** | 0.036 | 18.947 | 5.265 | 0.0152 | -1.818 |
| **10** | 0.037 | 19.473 | 2.635 | 0.0076 | -2.118 |
| **11** | 0.037 | 19.473 | 2.635 | 0.0076 | -2.118 |
| **12** | 0.036 | 18.947 | 5.265 | 0.0152 | -1.818 |
| **13** | 0.035 | 18.421 | 7.895 | 0.0233 | -1.632 |
| **14** | 0.033 | 17.368 | 13.160 | 0.0403 | -1.394 |

**2. Stress Degradation by Hydrolysis under 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 0.1N NaOH. Then, the volumetric flask was kept at normal condition for 90 minutes. After 15 min. time interval, 1 ml of solution was pipette out from this flask, diluted with 0.1N NaOH 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 cuvette.

For the blank, 0.5 ml solution of 3N HCl and 0.5 ml solution of 3N NaOH were diluted with 0.1 N NaOH in 10 ml of volumetric flask. After each 15 mins, again 1ml of the solution was pipetted out from the flask and the above procedure was repeated. The results were reported in Table 8.

**3. Stress Degradation by Hydrolysis under 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 0.1N NaOH. Volumetric flask was kept at normal condition for 90 min. After 15 min time interval, 1 ml of solution was pipette out from this flask, neutralized and diluted with 0.1N NaOH 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 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, each 15 mins 1ml of solution was again pippetted out from the flask and the above procedure was repeated.The results were reported in Table 8.

**4. Dry Heat Induced Degradation:** Pioglitazone sample was taken in a petriplate 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 0.1N NaOH in order to make the volume up to 10 ml (1000µg/ml). From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis. The results were reported in Table 8.

**5. 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 with0.1N NaOH. 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 normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on 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 analysed in UV. The results were reported in Table 8.

**6. Photolytic degradation:** Ten milligrams sample was dissolved in 0.1N NaOH and volume made up to 10 ml. From this solution appropriate dilution (30µg/ml) were made using 0.1N NaOH and 5 different dilutions were kept in sunlight. After 1hour 1st sample was taken in cuvette for the U.V analysis.

Similarly each sample was analysed every hour till 5 hrs. All the photolytic studies were carried out in the month of February. The results were reported in Table 8.

**Table 8: Stress Degradation Studies Result**

| Condition | Time | % Degradation |
|---|---|---|
| Acidic[0.1N NaOH(1ml) ] | 60 mins | 11.53% |
| | 90 mins | 21.15% |
| Alkaline[3N HCl(1ml)] | 60 mins | 5.76% |
| | 90 mins | 9.61% |
| Oxidative[30% Hydrogen Peroxide(1ml)] | 15mins | 19.23% |
| Dry Heat [ 70°] | 48hr | 1.93 % |
| Photolytic | 3 hr | 9.61 % |
| | 5 hr | 15.38% |

## RESULT AND DISCUSSION

Pioglitazone was freely soluble in Methanol, 0.1N NaOH and 0.1N HCl. Methanol was chosen as a solvent. The drug has maximum absorbance at 231.5 nm. The optical characteristic of drug was found to be Beer's law limits 15-65 µg/mL, Correlation coefficient is 0.9983.

The drug sample was analyzed by UV spectroscopy using 0.1N NaOH as solvent and the average content of drug present in the formulation was found to be 100.52%. The % RSD of accuracy studies was found to be less than 2%. The %RSD of precision was found to be 1.54% .The% RSD of ruggedness was found to be 0.73% to 0.88%.

The pH degradation studies of API were found less at 7-12. The force degradation studies of Pioglitazone tablet formulation was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 5.76% for 60min, 9.61% for 90min for API. Stress degradation by hydrolysis under acidic condition by using 3N HCl and product degradation was found to be11.53% for 60min and 21.15% for 90 min.

Dry heat induced degradation was done by using 70ºC temperature was found to be 1.93 % for 48 hrs. Oxidative degradation was done by using Hydrogen peroxide and product degradation was found to be 19.23% at 15 min. Photolytic degradation was found to be 9.61 % for 3hrs and 15.38% for 5 hrs for API.

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