Research Article

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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR PIOGLITAZONE DRUG SUBSTANCE BY REVERSE PHASE HPLC

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ABSTRACT

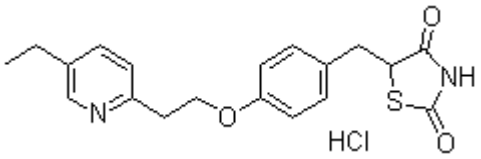
Pioglitazone is used for the treatment of diabetes mellitus type 2. A simple, precise cost effective and stability indicating Reverse Phase-HPLC method has been developed and validated for the determination of Assay of Pioglitazone Drug Substance. Separation of all known impurities from Pioglitazone was achieved with in shorter run time with required, accuracy and precision thus enabling the utility of the method for routine analysis. Chromatographic separation was achieved on a Prontosil C8 SH (250*4.6mm), 5µ using a mobile phase consisting of 550ml of pH 4.0 Phosphate buffer, 300ml Acetonitrile and 150ml of Methanol at a flow rate of 1.5 ml per minute. The detection was made at 254nm. The retention time of Pioglitazone peak is 5.9 minutes. The method was found linear over the range of 50 to 150%. The proposed method was validated as per the ICH and USP guidelines.

Key words: Pioglitazone, HPLC Method development and validation

INTRODUCTION:

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action. [1]. It is used for the treatment of diabetes mellitus type 2. Pioglitazone acts as an agonist at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPAR-gamma receptors increases the transcription of insulin-responsive genes involved in the control of glucose production, transport, and

utilization. In this way, pioglitazone both enhances tissue sensitivity to insulin and reduces hepatic gluconeogenesis. Thus, insulin resistance associated with type 2 diabetes mellitus is improved without an increase in insulin secretion by pancreatic β cells.



Name : Pioglitazone hydro0.chloride

Synonyms : [5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-] thiazolidinedione hydrochloride

Molecular Formula : C19H20N2O3S.HCl

Molecular Weight : 392.90

Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α.[2][3] It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue, and the liver. As a result, pioglitazone reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose, insulin and glycated hemoglobin in the bloodstream. Although not clinically significant, pioglitazone decreases the level of triglycerides and increases that of high-density lipoproteins (HDL) without changing low-density lipoproteins (LDL) and total cholesterol in patients with disorders of lipid metabolism, although statins are the drug of choice for this. [4][5] Pioglitazone is used for the treatment of diabetes mellitus type 2 (previously known as non-insulin-dependent diabetes mellitus, NIDDM) in monotherapy and in combination with a sulfonylurea, metformin, or insulin. Pioglitazone has also been used to treat non-alcoholic steatohepatitis (fatty liver), but this use is presently considered experimental.[6] Pioglitazone has also been found to reduce the risk of conversion from prediabetes to diabetes mellitus type 2 by 72%. [7] Pioglitazone is currently being reviewed. A meta-analysis released subsequently showed that pioglitazone reduced the number of ischemic cardiac events rather than increase the risk, but increases CHF.[8] Chronic administration of the drug has led to occasional instances of cholestatic hepatitis, reversible upon drug discontinuation.[9] On June 9, 2011 the

French Agency for the Safety of Health Products decided to withdraw pioglitazone in regards to high risk of bladder cancer [10] On June 10, 2011 Germany's Federal Institute for Drugs and Medical Devices also advised doctors not to prescribe the medication until further investigation of the cancer risk had been conducted.[11] On June 15, 2011 the U.S. FDA announced that pioglitazone use for more than one year may be associated with an increased risk of bladder cancer, and that the information about this risk will be added to the Warnings and Precautions section of the label for pioglitazone-containing medicines. The patient Medication Guide for these medicines will also be revised to include information on the risk of bladder cancer.[12]

MATERIALS AND METHODS

I. Chemicals and Reagents:

Pioglitazone working standard, Water (Milli Q), Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Potassium dihydrogen orthophosphate (AR Grade), Triethylamine (AR Grade) AND *Ortho* Phosphoric Acid (88%, AR Grade).

II. Apparatus and Chromatographic Conditions:

HPLC analysis was performed on Waters HPLC system with diode array detector. Separations were carried on a Prontosil C8 SH (250*4.6mm), 5µ) using isocratic elution. The flow rate was 1.5 mL min-1. UV detection was performed at 254 nm. HPLC Column temperature was 40°C. Peak identity was confirmed by retention time comparison and the HPLC was operated at room temperature.

III. Preparation of Mobile Phase and Diluent:

Mobile Phase: Mix thoroughly 550 mL of 4.0 pH Phosphate buffer with 300 mL of filtered Acetonitrile and 150 mL of filtered Methanol.

Diluent: Mix Water and Methanol in the ratio of 1:1

IV. Preparation of Standard Solution:

Accurately weigh and transfer about 50 mg of Pioglitazone working standard into a 50 mL volumetric flask, dissolve in and dilute to volume with Methanol. Dilute 5 mL of this solution to 50 mL with diluent.

V. Preparation of Sample Solution:

Accurately weigh and transfer about 50 mg of sample into a 50 mL volumetric flask, dissolve in and dilute to volume with Methanol. Dilute 5 mL of this solution to 50 mL with diluent.

RESULTS AND DISCUSSION

Method Development

Chromatographic parameters were preliminary optimized to develop a stability indicating Assay method for Pioglitazone with short analyses time (<10 min). Since Pioglitazone is having three impurities. So these impurities need to separate from main analyte to show the stability indicating Assay method. I have tried solubility of the drug with different buffers and found that Potassium dihydrogen orthophosphate was suitable. The development trials were initiated with the selection of mobile phase. Since the opted development method is Reverse phase, various polar solvents and buffers (Solvents such as Acetonitrile, Methanol andEthanol etc.) were used in the initial developmental trials and concluded with the efficient mobile phase i.e. mixture of Phosphate buffer, Acetonitrile and Methanol. Various compositions of the selected solvents were tried on different columns available such as Symmetry, ACE, Phenomenex and Hypersil BDS etc.

With the better resolution and peak shape the method was optimized by the mobile phase composition of Phosphate buffer (pH 4.0), Acetonitrile and Methanol in the ratio of 550:300:150 on Prontosil C8 SH (250*4.6mm), 5µ. System Suitability parameters were evaluated and limits fixed. USP Tailing factor and %RSD of five injections for Pioglitazone standard performed and found that within the limits

Method Validation

The above method was validated according to ICH and USP guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method [13].

System Suitability

In order to determine the reproducibility of the proposed methodology, suitability parameters including Retention Time, USP Tailing factor, and %RSD of Pioglitazone peak areas were investigated. The results are summarized in **Table 1.**

Specificity

Interference from Blank:

The specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix. Specificity was evaluated by injecting the blank solution to observe for interference at the retention times of all known impurities and principle peak. It was observed that there was no interference from the blank solution. The Blank and Sample

chromatograms were shown in figure- **1-2**.

Interference from Impurities:

All known impurities were injected individually and spiked into test at specification level and injected in to the system. All the impurities were well separated from main analyte. The Spiked chromatogram was shown in **Figure- 3**.

Forced degradation Studies:

Drug Substance subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analyzed for peak purity of Pioglitazone peaks using Empower software. For all stressed samples the peak purity of Pioglitazone was found within the limits. The results are summarized in **Table 2** and degradation chromatograms are shown in **figure-4-6**.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Six replicate samples were prepared and analyzed as per the test procedure. The % Relative standard deviations for Assay of Pioglitazone calculated and the results are found to be within the acceptance criterion. The results are summarized in **Table 3**

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy may be inferred once precision, linearity and specificity have been established.

Linearity of Detector Response:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detector response for Pioglitazone was established by analyzing a series of solutions of Pioglitazone at the concentration ranging from 50% to 150% level of test concentration were prepared and injected into the HPLC system. The final concentration of each solution in µg per mL was plotted against peak area

response. Slope, correlation coefficient (R) and intercept were found to be within the limit. The results are shown in **Table 4.**

Robustness:

Robustness of the method was verified by deliberately varying the following conditions. by changing the flow rate by ± 10%. By changing the column oven temperature by ± 5°C. By changing the organic content in mobile phase by ± 2% absolute.

Standard Solution and test solutions were prepared as per the test procedure and analysed in each varied condition. System suitability parameters and RRT of all known impurities were evaluated with each varied condition and compared with test method conditions was found to be within the limit.

Ruggedness:

Bench Top Stability of Test Solution:

Bench top stability of test solution of Pioglitazone drug substance was conducted over a period of 2 days and found that test solution is stable on Bench top for 2 days.

Refrigerator Stability of test solution:

Refrigerator stability of test solution of Pioglitazone drug substance was conducted over a period of 2 days and found that test solution is stable in refrigerator for 2 days.

Bench Top Stability of Mobile Phase:

Bench top stability of mobile phase was conducted over a period of 2 days and found that mobile phase is stable on Bench top for 2 days.

CONCLUSIONS:

A simple, rapid, cost effective and accurate Reverse Phase-HPLC method was developed for the Stability indicating Assay method for Pioglitazone drug substanc. The HPLC method was validated and demonstrated that good linearity, precision, accuracy, specificity and stability indicating capacity thus, the developed HPLC method can be utilized for routine analysis and stability studies for Pioglitazone Drug Substance.

Figure 1: Chromatogram of Blank

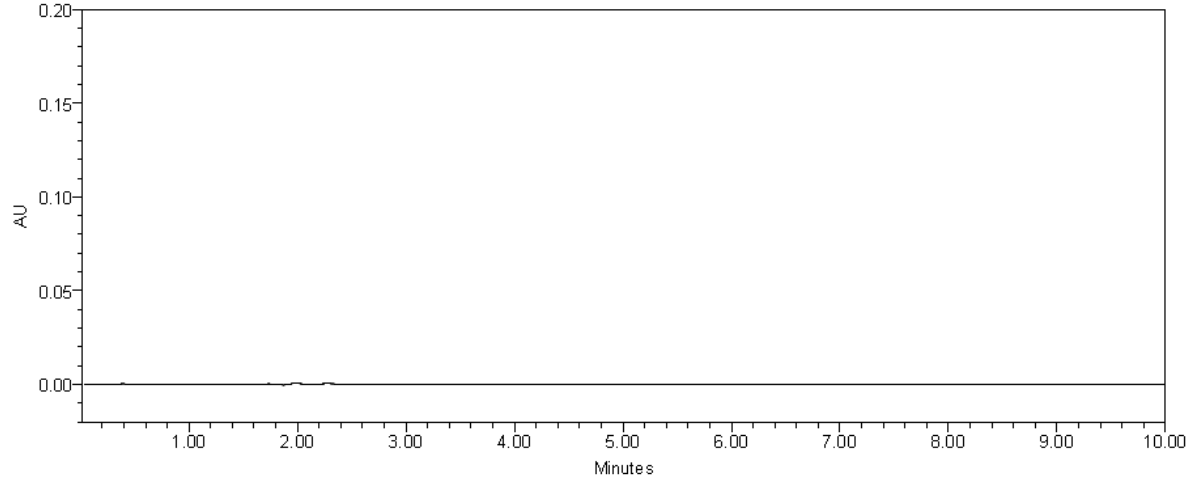
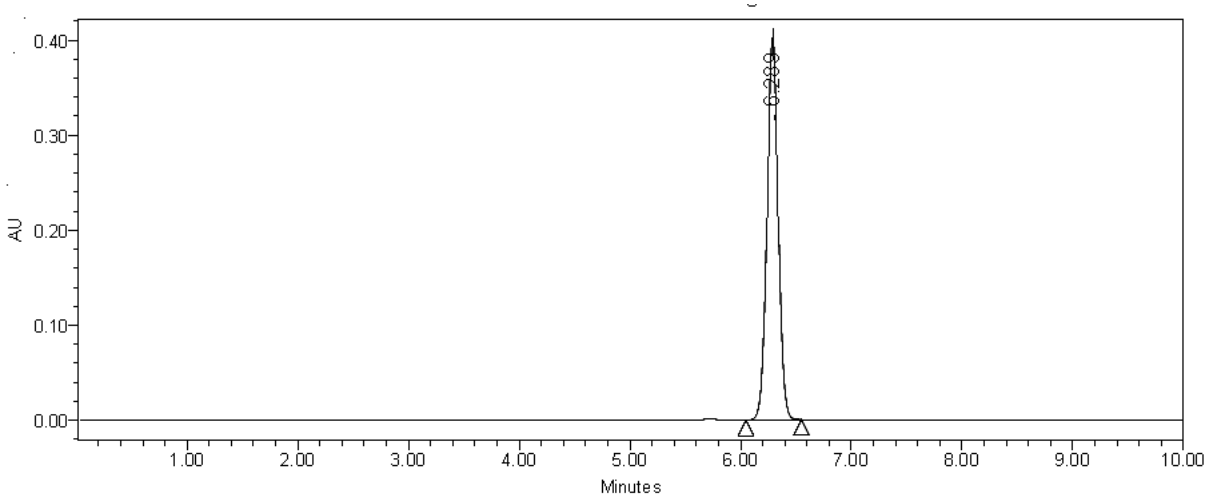


Figure 2: Chromatogram of Sample



Peak Table:

S.No.	Name of the peak	Retention time
1	Pioglitazone	6.289

Peak Table:

S.No.	Name of the peak	Retention time
1	Pioglitazone	6.216

Figure 3: Chromatogram and Purity Plot of Pioglitazone Drug Substance spiked with related impurities.

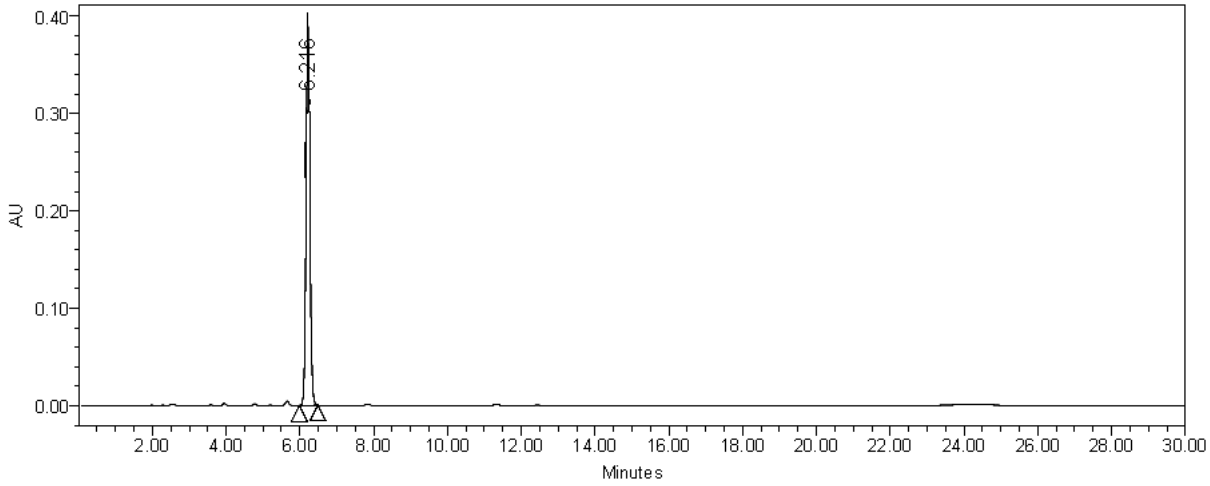
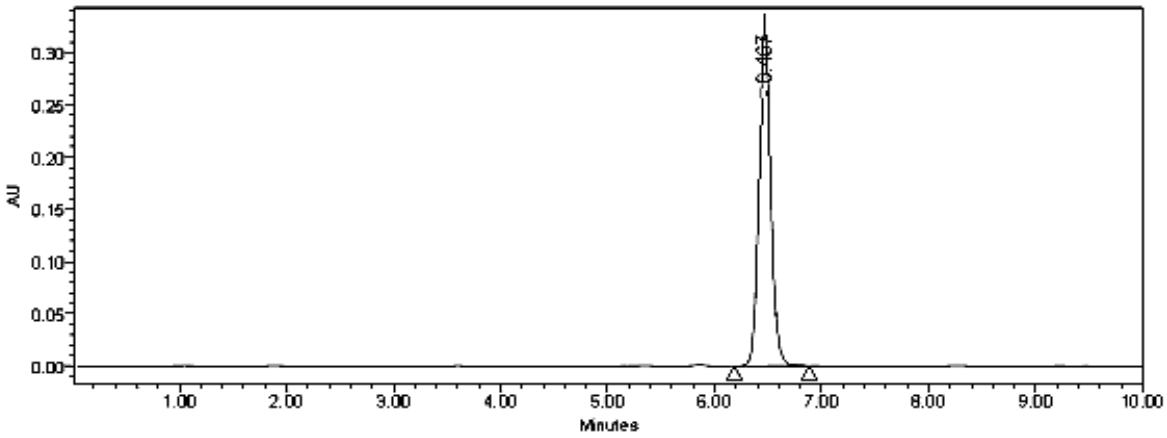


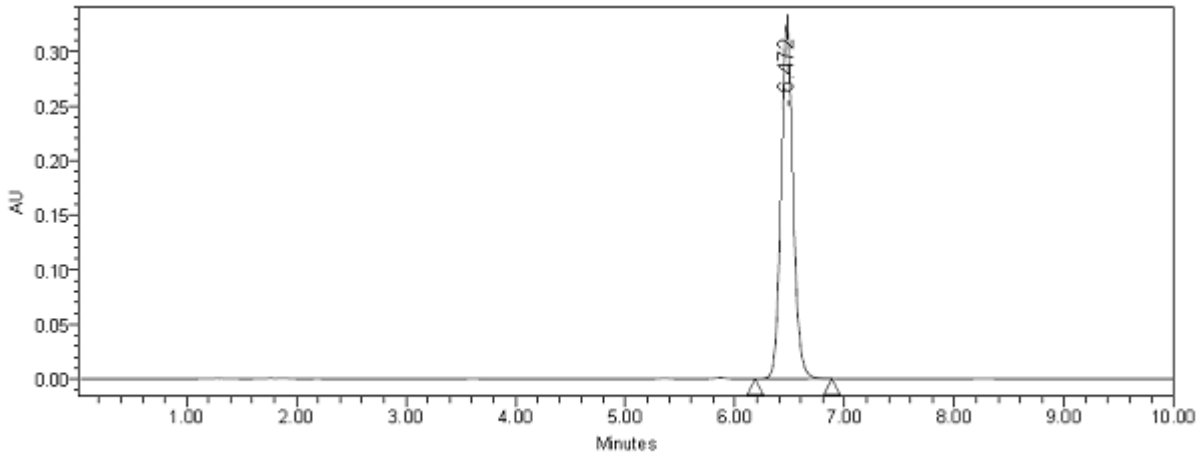
Figure 4: Chromatogram and Purity Plot of Heat Stressed Pioglitazone Drug Substance



Peak Table:

S.No.	Name of the peak	Retention time
1	Pioglitazone	6.467

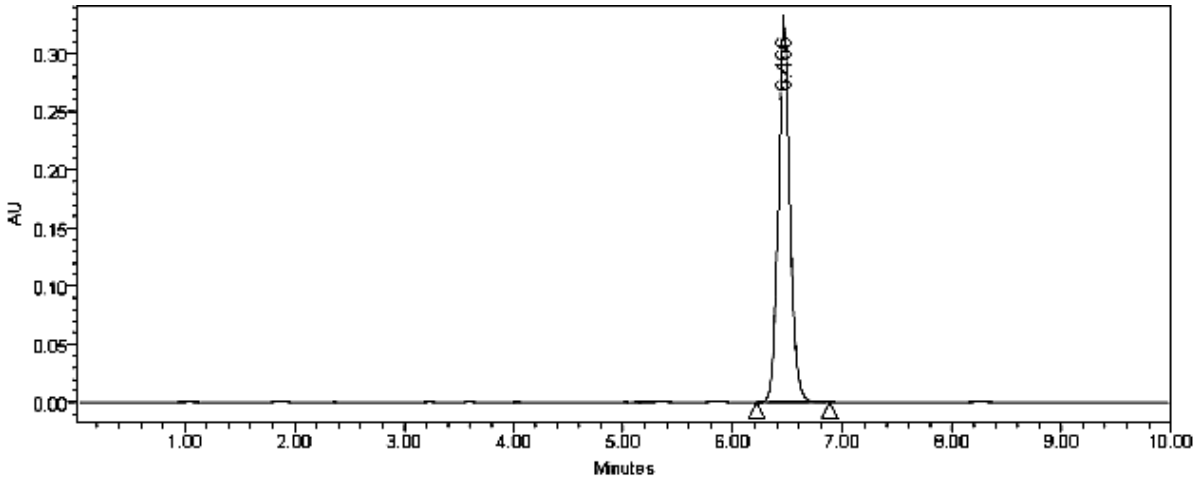
Figure 5: Chromatogram and Purity Plot of Humidity Stressed Pioglitazone Drug substance



Peak Table:

S.No.	Name of the peak	Retention time
1	Pioglitazone	6.472

Figure 6: Chromatogram and Purity Plot of Photolytically Stressed (UV Light) Pioglitazone Drug Substance



Peak Table:

S.No.	Name of the peak	Retention time
1	Pioglitazone	6.466

Figure 7: Linearity plot of Pioglitazone

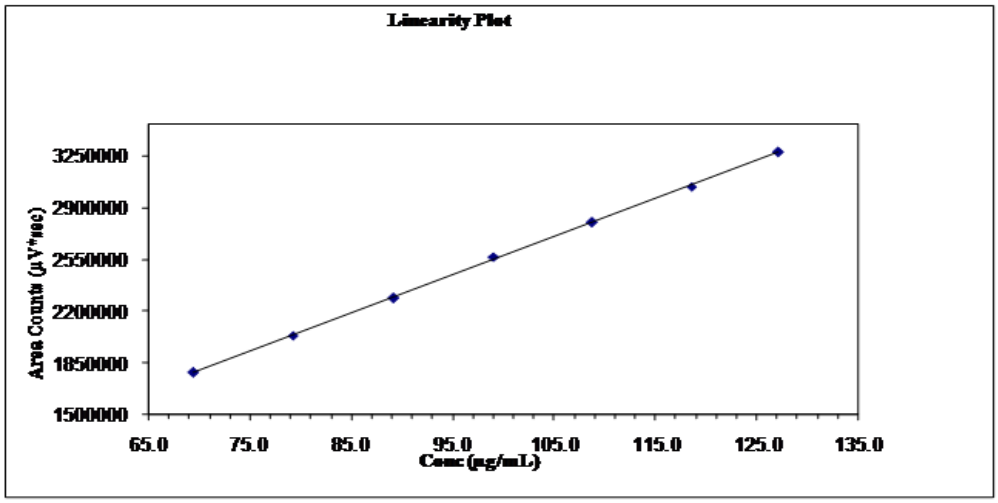


Table1: System Suitability

System Suitability Parameters	Observations	Acceptance Criteria
USP Tailing factor for PIOGLITAZONE peak in standard solution	1.0	NLT 0.85 & NMT 2.0
% RSD of area counts of PIOGLITAZONE Peak from five replicate injections of Standard solution	0.16	Not more than 1.0

Table: 2 Forced Degradation Data

Pioglitazone Assay - Forced Degradation				
Condition	**%Degradation**	**Purity Angle**	**Purity Threshold**	**Purity Flag**
Exposed to heat at 105°C for 358 hours	0	0.120	1.089	No
Stressed with Humidity 25°C/97% RH for 358 hours	0	0.113	1.077	No
Exposed to UV light for 358 hours (432.0 watts hours/m^2).	0	0.112	1.102	No

Table 3: Precision

S. No.	Assay (%w/w, on Anhydrous and Solvent free basis)
1	99.43
2	99.63
3	99.46
4	98.75
5	98.66
6	98.89
Average	99.14
%RSD	0.42

Table: 4 Linearity Data

S.No.	Concentration (µg/mL)	Average area (µV*Sec)
1	69.46	1786390
2	79.24	2034971
3	89.18	2285693
4	99.06	2567223
5	108.72	2797797
6	118.54	3041722
7	127.12	3270833
SLOPE		**25742**
STEYX		**10015**
INTERCEPT		**-1610**
Correlation coefficient		**0.99986**

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