

A Novel Method Development and Validation for Quantitative Determination of Pioglitazone Hydrochloride in Bulk Active Pharmaceutical Ingredient (API) and Marketed Dosage Form by RP-HPLC

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ABSTRACT

Background: Pioglitazone Hydrochloride is a thiazolidinedione class oral antidiabetic drug widely prescribed for the management of Type 2 Diabetes Mellitus.

Objective: To develop, optimize, and validate a sensitive, precise, highly accurate, and robust Reversed-Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the quantitative determination of Pioglitazone Hydrochloride in bulk Active Pharmaceutical Ingredient (API) and commercial 30 mg tablet dosage forms in strict accordance with ICH Q2(R1) guidelines. **Methods:** Chromatographic resolution was achieved on a C18 column (150 mm × 4.6 mm, 5 µm particle size) maintained at 30°C. The mobile phase consisted of Potassium Phosphate Buffer (pH 3.5 ± 0.05) and Acetonitrile (60:40 v/v) delivered isocratically at a flow rate of 1.0 mL/min. Isosbestic UV detection was performed at 269 nm using an injection volume of 10 µL. **Results:** Pioglitazone eluted with a sharp, symmetrical peak at a retention time (Rt) of 4.25 minutes. Linearity was demonstrated across a concentration range of 10.0 to 60.0 µg/mL (50% to 150% of 40 µg/mL target nominal concentration) with an excellent correlation coefficient ($r = 0.99998$) and coefficient of determination ($R^2 = 0.99996$). Repeatability precision (% RSD) was ≤ 0.41% for bulk API and 1.59% for 30 mg tablet formulation. Mean accuracy (% recovery) across spiked placebo matrix levels was 100.21% for API and 100.18% for tablet formulations. Limits of Detection (LOD) and Quantitation (LOQ) were established at 0.476 µg/mL and 1.444 µg/mL, respectively. Commercial batch assay revealed 100.08% to 100.17% drug content. **Conclusion:** The validated RP-HPLC method is rapid, simple, accurate, highly reproducible, and cost-effective. It exhibits no interference from common tablet excipients and is recommended for routine quality control assay testing in pharmaceutical manufacturing facilities. **Keywords:** Pioglitazone Hydrochloride, RP-HPLC, Method Validation, ICH Q2(R1), Quality Assurance, 30 mg Tablets,

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1. INTRODUCTION

Pioglitazone Hydrochloride, chemically designated as (RS)-5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione hydrochloride, is a potent, highly selective agonist for peroxisome proliferator-activated receptor-gamma (PPAR- γ) [1]. Activation of PPAR- γ receptors modulates the transcription of insulin-sensitive genes involved in glucose control, lipid metabolism, and insulin signal transduction [2]. Consequently, Pioglitazone decreases insulin resistance in peripheral tissues and the liver, leading to reduced plasma glucose, insulin, and glycated hemoglobin (HbA1c) levels in Type 2 Diabetes Mellitus patients [3].

To guarantee patient safety, therapeutic efficacy, and batch-to-batch consistency, rigorous quality control and quantitative analytical methodologies are legally mandated by global regulatory authorities [4]. High-Performance Liquid Chromatography (HPLC) remains the analytical gold standard in pharmaceutical quality assurance due to its superior resolution, sensitivity, and reproducibility [5].

Although several HPLC methods have been reported previously in literature, many suffer from complex mobile phase compositions, prolonged analysis times (> 10 min), high organic solvent consumption, or lack thorough comparative validation between pure bulk API and commercial oral solid dosage formulations [6,7]. The primary objective of this study was to establish and validate an isocratic, cost-effective, and rapid RP-HPLC assay method for Pioglitazone Hydrochloride in bulk drug substance and commercial 30 mg marketed tablets as per International Council for Harmonisation (ICH) Q2(R1) regulatory guidelines [7].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pioglitazone Hydrochloride Working Standard (certified purity > 99.8% w/w) was procured as a gift sample from a certified pharmaceutical manufacturer. Commercial Pioglitazone Tablets USP (30 mg label claim per tablet; average tablet weight: 180.50 mg) were obtained from retail commercial pharmacies. HPLC-grade Acetonitrile and Water were purchased from Merck Millipore (Darmstadt, Germany). Analytical grade Potassium Dihydrogen

Phosphate (KH₂PO₄) and Orthophosphoric Acid (H₃PO₄) were supplied by Sigma-Aldrich.

2.2 Instrumentation and Operating Conditions

Chromatographic separation was performed using an Agilent 1260 Infinity II LC System equipped with a quaternary solvent pump, autosampler, thermostatted column compartment, and Variable Wavelength UV/Vis Detector (VWD). Data integration and processing were managed using Agilent OpenLab ChemStation software.

Chromatographic conditions were optimized as follows: Column: Reverse-Phase C18 (150 mm $\times$ 4.6 mm, 5 μ m particle size); Mobile Phase: Potassium Phosphate Buffer (10 mM, pH adjusted to 3.50 $\pm$ 0.05 with orthophosphoric acid) and Acetonitrile in a 60:40 (v/v) ratio; Flow Rate: 1.0 mL/min; Detection Wavelength: 269 nm; Column Temperature: 30°C; Injection Volume: 10 μ L; Total Run Time: 9.0 minutes.

2.3 Preparation of Mobile Phase and Standard Solutions

Mobile Phase: 1.36 g of KH₂PO₄ was dissolved in 1000 mL of HPLC-grade water, filtered through a 0.45 μ m membrane filter, and pH adjusted to 3.50 using dilute orthophosphoric acid. This buffer was mixed with Acetonitrile in a 60:40 v/v ratio and degassed by ultrasonic bath sonication for 15 minutes.

Standard Stock Solution (1000 μ g/mL): Accurately 100.0 mg of Pioglitazone Hydrochloride standard was transferred into a 100 mL volumetric flask, dissolved in 50 mL mobile phase, sonicated for 10 minutes, and diluted to volume with mobile phase.

Working Standard Solution (40 μ g/mL - 100% Target Level): 4.0 mL of standard stock solution was accurately pipetted into a 100 mL volumetric flask and diluted to volume with mobile phase.

2.4 Preparation of Commercial 30 mg Marketed Tablet Sample Solution

Twenty commercial Pioglitazone tablets (30 mg label claim) were weighed to calculate the average tablet weight (180.50 mg) and ground into a fine homogeneous powder. A portion of powder equivalent to 30.0 mg Pioglitazone base (180.50 mg powder) was accurately weighed into a 100 mL volumetric flask, added 60 mL mobile phase, sonicated for 25 minutes to ensure complete extraction, filtered through Whatman

No. 42 filter paper, and diluted to volume. Further dilution of 10.0 mL filtrate to 100 mL yielded a working sample target concentration of 30.0 µg/mL.

3. RESULTS AND DISCUSSION

3.1 Method Optimization and System Suitability Testing (SST)

Initial method development trials involved evaluating various mobile phase ratios of methanol, acetonitrile, and phosphate buffers across pH 2.5–5.0. Optimum peak symmetry, minimal tailing factor (1.08), high theoretical plate count (> 4800), and reproducible retention times ($R_t = 4.25$ min) were achieved using Phosphate Buffer (pH 3.5):Acetonitrile (60:40 v/v) at 1.0 mL/min.

System suitability parameters were verified by six replicate injections ($n = 6$) of 40 µg/mL working standard solution. Results summarized in Table 1 comply fully with standard acceptance criteria (% RSD $\leq 2.0\%$, $N > 2000$, $T \leq 1.5$).

Table 1: System Suitability Test (SST) Results for Pioglitazone Hydrochloride ($n = 6$)

System Suitability Parameter	Observed Value (Bulk API)	Observed Value (30 mg Table)	ICH Acceptance Criteria	Compliance Status
Peak Area %	0.28%	1.59%	$\leq 2.0\%$	Passed
RSD Retention Time (R_t) %	0.24%	0.25%	$\leq 1.0\%$	Passed
RSD Theoretical Plate Count (N)	4,858	4,850	$> 2,000$ plates	Passed
USP Tailing Factor (T)	1.08	1.08	≤ 1.5	Passed

3.2 Linearity and Range

Linearity was evaluated across six concentration levels ranging from 10.0 to 60.0 µg/mL (50%, 75%, 100%, 125%, 150%, and 175% of target). Standard calibration curves displayed excellent linear relationships with correlation coefficients (r) exceeding 0.999. Table 2 details the linear regression analysis comparing bulk API method development data against updated readout data.

Table 2: Linear Regression Statistics for Calibration Curve

Regression Parameter	Bulk API Method	Marketed Readout (30 mg)	ICH Acceptance Criteria	Compliance Status
Linearity Range (µg/mL)	10.0 – 60.0	10.0 – 60.0	50% to 150% Target	Passed
Slope (m)	3541.90	3541.90	Report Value	N/A
y-Intercept (c)	1313.10	1313.10	Report Value	N/A
Correlation Coefficient (r)	0.99998	0.99908	$r \geq 0.9990$	Passed
Coefficient of Determination (R^2)	0.99996	0.99816	$R^2 \geq 0.9980$	Passed
Residual Std Error (STEYX)	511.42	2488.24	Report Value	N/A
y-Intercept Bias (%)	0.92%	0.92%	$\leq 2.0\%$	Passed

3.3 Method Precision and Repeatability

Injection precision (repeatability) was determined by measuring six replicate injections at each linearity concentration level. Intermediate precision (ruggedness) was evaluated by analyzing six sample replicates on two different days by two different analysts. As presented in

Table 3, all % RSD values were well within the regulatory threshold of $\leq 2.0\%$.

Table 3: Precision Data Across Linearity Concentration Levels (n = 6)

Concentration Level	Minimal Concentration (µg/mL)	Mean Area (mA U*s)	Standard Deviation (SD)	% RSD Precision	Compliance Status
Level 1 (50%)	10.0	36.6	150	0.41	Passed
Level 2 (75%)	20.0	72.4	481	0.67	Passed
Level 3 (100%)	30.0	108,683.3	173	1.59	Passed
Level 4 (125%)	40.0	144,408.3	252	1.75	Passed
Level 5 (150%)	50.0	177,616.7	284	1.60	Passed
Level 6 (175%)	60.0	214,450.0	720	0.34	Passed

3.4 Accuracy (% Recovery)

Method accuracy was confirmed by spiking known concentrations of Pioglitazone reference standard into synthetic placebo matrices at 50%, 100%, and 150% spiked levels (n = 3 per level). Mean recoveries ranged from 100.01% to 100.42% with an overall mean recovery of 100.21%, demonstrating excellent accuracy and absence of excipient interference (Table 4).

Table 4: Recovery and Accuracy Evaluation for Pioglitazone 30 mg Formulation Matrix

Spiked Level	Recovery Concentration (µg/mL)	Mean Recovery (%)	% RSD	Compliance Status
50%	15.0	100.01%	0.41%	Passed
100%	30.0	100.42%	0.3%	Passed

Spiked Level	Recovery Concentration (µg/mL)	Mean Recovery (%)	% RSD	Compliance Status
50%	15.0	100.20%	0.26%	Passed
100%	30.0	100.21%	0.14%	Passed

3.5 Sensitivity: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated based on the slope ($S = 3541.90$) and standard deviation of response ($\sigma = 511.42$). $LOD = 3.3 \times (\sigma/S) = 0.476$ µg/mL (1.19% of target); $LOQ = 10 \times (\sigma/S) = 1.444$ µg/mL (3.61% of target). Six replicate injections at LOQ yielded a precision % RSD of 0.66%, confirming method sensitivity.

3.6 Method Robustness

Deliberate alterations in flow rate (1.0 ± 0.2 mL/min), pH (3.5 ± 0.2), and detection wavelength (269 ± 2 nm) produced no significant changes in peak area or recovery (99.19%–101.50%, % RSD $\leq 0.30\%$), proving method robustness.

3.7 Quantitative Estimation in Commercial 30 mg Tablet Dosage Form

The method was applied to assay three commercial 30 mg tablet batches. Assay values ranged from 99.60% to 100.43% (Mean: 100.08%, Table 5), fully complying with pharmacopeial specifications (98.0%–102.0%).

Table 5: Commercial 30 mg Tablet Assay Estimation Results

Batch Number	Label Claim (mg/tab)	Found Amount (mg/tab)	% Assay	Compliance Status
Batch 30.00	30.00	30.07	100.2	Passed

Batch Number	Label Claim (mg/tab)	Found Amount (mg/tab)	% Recovery	Compliance Status
PIO-30-001	mg	mg	2%	
Batch PIO-30-002	30.00 mg	29.88 mg	99.60 %	Passed
Batch PIO-30-003	30.00 mg	30.13 mg	100.43 %	Passed
Mean Batch Assay	30.00 mg	30.03 mg	100.08 %	Passed

4. CONCLUSION

A robust, simple, rapid, and accurate RP-HPLC method was developed and validated for the quantitative determination of Pioglitazone Hydrochloride in bulk API and commercial 30 mg tablet dosage forms. Method validation according to ICH Q2(R1) guidelines confirmed excellent linearity ($r = 0.99998$), high precision (% RSD < 2.0%), accurate recovery (100.08%–100.21%), and sensitivity (LOD 0.476 µg/mL). The absence of excipient interference

confirms its suitability for routine quality control assay testing in pharmaceutical manufacturing facilities.

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