WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 11, 2601-2012. Review Article ISSN 2277–7105

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PIOGLITAZONE HYDROCHLORIDE BY RP-HPLC AND ITS APPLICATION IN BULK AND PHARMACEUTICAL DOSAGE FORM

Dr. Ch. Saibabu*[1], P. Narasimha Naidu[2], Dr. M. Bhaskar[3]

[1]*Professor, Department of Pharmaceutics, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

[2]Department of Pharmacy, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

[3]Professor, Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

Article Received on 05 May 2026,
Article Revised on 25 May 2026,
Article Published on 03 June 2026,

https://doi.org/10.5281/zenodo.20537215

***Corresponding Author**

Dr. Ch. Saibabu

Professor, Department of Pharmaceutics, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

How to cite this Article: Dr. Ch. Saibabu*[1], P. Narasimha Naidu[2], Dr. M. Bhaskar[3] (2026). Method Development And Validation For The Estimation Of Pioglitazone Hydrochloride By Rp-Hplc And Its Application In Bulk And Pharmaceutical Dosage Form. World Journal of Pharmaceutical Research, 15(11), 2601-2012.

ABSTRACT

A simple, precise and stability-indicating HPLC method was developed and validated for the simultaneous determination of anti-diabetic drugs. The separation was achieved on ACE 3 150mm x 4.6mm, 3.5µm column with gradient flow. The mobile phase at a flow rate of 1.5 mL min−1 consisted of 10mM sodium hexane sulphonate monohydrate and 10mM Potassium dihydrogen phosphate buffer with acetonitrile and methanol in gradient ratio. The UV detection was carried out at 210 nm. The method was successfully validated in accordance to ICH guidelines. Further, the validated method was applied for commercially available pharmaceutical dosage form.

KEYWORDS: Anti-diabetic drugs, Validation, HPLC, Analysis, ICH.

INTRODUCTION

Pioglitazone an insulin sensitizer,S1] chemically a (RS)-5-(4-

[2-(5-ethylpyridin-2-yl)ethoxy] benzyl)thiazolidine-2,4-dione an type 2 oral antdiabetic drug, sold under brand name Actoplus Met, Actos, Duetact, Glidipion, etc. Type 2 diabetes where the patients lack the capability of producing enough insuin in the body.[1,2] Pioglitazone activates a ligand-activated transcription factor PPAR-gamma, inducing cell differentiation and inhibiting cell growth and angiogenesis.[3] Pioglitazone inhibits macrophage and monocyte activation, adapts the transcription of insulin responsive genes, and stimulates adipocyte differentiation.[4,5] Pioglitazone enhances insulin sensitivity by making cells more responsive to it. In patients with type 2 diabetes, pioglitazone improves glycemic control mostly through enhancing peripheral insulin sensitivity, whereas metformin lowers hepatic glucose output. In hypoglycemic situations, pioglitazone masks symptoms such as increased heart rate, dizziness, and perspiration. It also has side effects such as edoema (when used with a sulfonylurea or insulin), heat failure, and respiratory infection. Due to the risk of urinary bladder cancer, pioglitazone was banned as an anti-diabetic medicine on July 18, 2013, however the prohibition was reversed on July 31, 2013.[6,7] Literature survey reveals that, analytical and bio-analytical methods have been developed and validated for the estimation of Pioglitazone in bulk, pharmaceutical formulation and biofluids, which include techniques like HPLC, Spectrophotometry, and Polarography.[8-12] The current study has been undertaken to develop RP-HPLC method for the determination of Pioglitazone in bulk and pharmaceutical formulation.

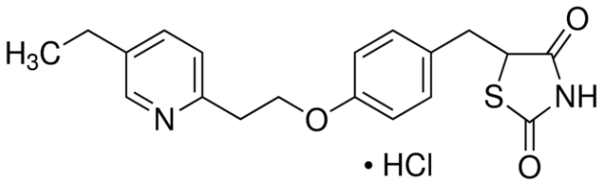

Figure 1.0. Chemical structure of Pioglitazone. Hcl.

MATERIALS AND METHODS

Chemicals and reagents

Drug substances were provided by Getz Pharma Research Pvt. Ltd, India. All the chemicals and reagents Ammonium hydroxide, hydrochloric acid, potassium dihydrogen phosphate, hydrogen peroxide (50 %) were used of Analytical grade. While, Acetonitrile and Methanol was procure from Merck (Germany). A Millipore Milli Q plus water purification system (Milford, USA), was used to prepare distilled water (conductivity >18 μΩ). The commercially available drug products were used as Jalara (Vildagliptin tablets, 50mg, Novartis Pharma) and Pioz30 (Pioglitazone HCl Tablets, 30mg, USV Limited).

Instruments

Integrated HPLC system, manufactured by Waters (USA) was used for method development and method validation. This system comprised of a quaternary gradient pump, auto sampler, column oven and a photodiode array detector. PC installed Empower was used to record and integrates the chromatograms. The analysis was carried out at ambient temperature. Photostability studies were performed in a photostability chamber, from Thermolab (India).

Preparation of standard Stock solution

Weigh accurately about 10mg of working standard and transfer to 100ml volumetric flask. Then make up the volume with methanol and shake it till it get dissolved and sonicate the solution ultrasonicater to degas the solution prepared.

Final Preparation of standard solution: (Pioglitazone hydrochloride 0.01mg/ml)

From the above standard stock solution, 1ml of solution was pipetted and transferred into 10ml volumetric flask. Then make up the volume with methanol and shake it till it get dissolved and sonicate the solution ultrasonicater to degas the solution prepared.

Test Stock preparation

Weigh accurately about 10mg of sample and transfer to 100ml volumetric flask. Then make up the volume with methanol and shake it till it get dissolved and sonicate the solution in ultrasonicater to degas the solution prepared.

Final Test preparation: (Pioglitazone hydrochloride 0.01mg/ml)

From the above test stock solution, 1ml of solution was pipetted and transferred into 10ml volumetric flask. Then make up the volume with methanol and shake it till it get dissolved and sonicate the solution ultrasonicater to degas the solution prepared.

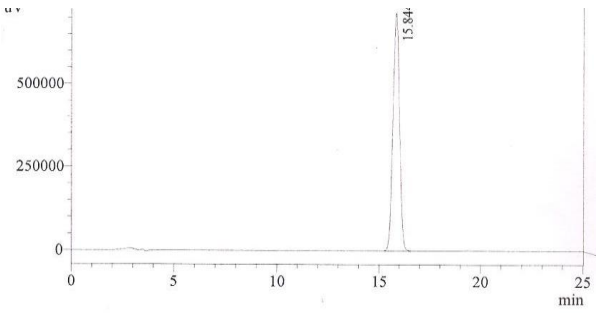

Figure 2.0: Chromatogram of Pioglitazone.

METHOD VALIDATION

SYSTEM SUITABILITY

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, HPLC method and the procedure should be capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

Procedure

- The standard solution and sample solution are prepared as per the assay method of concentration 10µg/ml and filter it through Millipore filter and sonicate.
- Carried out the system suitability studies for Standard solution and sample solution with a minimum of six replicates of single preparation.
- Calculate %RSD for area and retention time of Pioglitazone Hydrochloride, and record the tailing factor & theoretical plate count details were given in table no.7.1(a) and 7.1(b).
- Resulted chromatogram was shown in the chromatogram fig. no.7.1.1 and fig.no.7.1.2 for standard and sample respectively.

SPECIFICITY (SELECTIVITY)

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications.

Identification: To ensure the identity for an analyte.

Purity Tests: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Procedure

Individually inject 20µl of each solution individually the prepared solutions of standard and sample and develop chromatograms check the retention time of individual injection sample in the chromatogram and observe whether the retention time is matching with that of standard.

Placebo Interference

A study to establish the interference of placebo was conducted. Samples were prepared in triplicate by taking the placebo equivalent to about the weight in portion of test preparation as per the test method. Chromatogram of placebo did not show any additional peaks. This indicates that the excipients used in the formulation do not interfere in the assay of Pioglitazone Hcl tablets.

PRECISION

Precision is the measure of the degree of repeatability of analytical method under normal operation and is normally expressed as % RSD for the statistically significant number of samples.

SYSTEM PRECISION

The standard solution was prepared as per the assay method of concentration 0.05mg/ml and filter it through Millipore filter and sonicate.

Procedure

- Carried out the system precision for Standard solution with a minimum of six replicates of single preparation.
- Calculate %RSD for peak area and retention time of Pioglitazone Hydrochloride.
- The data for system precision was given in table no.1.

Observation: % RSD of the Pioglitazone Hcl tablet from six units was found to 0.67.

Table 1: Data for System Suitability.

Standard Solution				
S.NO.	**Retention Time**	**Area**	**Tailing Factor**	**Theoretical Plates**
1	8.766	11068663	1.024	9840.616
2	8.774	11068953	1.025	9814.033
3	8.774	11063195	1.025	9824.863
4	8.774	11064105	1.025	9860.318
5	8.775	11057067	1.025	9810.710
% RSD	0.05	0.04		

METHOD PRECISION

Six sample preparations were prepared individually using single batch of Pioglitazone Hcl as per assay method and injected each solution. Resulted data was shown in the fig. no.7.3.2(a).

Procedure

- Carried out the method precision studies for Pioglitazone hydrochloride at 100% concentration of the test solution with a minimum of six determinations of individual preparations.
- Calculated %RSD for Pioglitazone hydrochloride area and RT.

Acceptance criteria: The % RSD of Pioglitazone Hcl tablet from the six units should be not more than 2.0 %.

Observation: % RSD of the Pioglitazone Hcl tablet from six units was found to be 0.11.

ACCURACY (% RECOVERY).

Accuracy of test method was carried out by spiking known amount of drug substance to get concentration of Pioglitazone Hcl 80 %, 100 % and 120 % of target concentration in triplicate for each level. Each solution was injected in triplicate. The average % recovery of Pioglitazone Hcl was calculated.

Acceptance criteria: The mean % recovery of the Pioglitazone Hcl at each level should be in the range of 99.0 – 101.0%.

Observation: The recovery results indicating that the test method has an acceptable level of accuracy shown in table no: 4.

Table 4: Accuracy data for Pioglitazone Hcl.

Concentration of Spiked Sample	Amount added(ppm)	Amount found(ppm)	% Recovery	Statistical Analysis of % Recovery	
80% Sample	16	15.96	99.75	Mean	99.68
	16	15.85	99.06	SD	0.5970
	16	16.04	100.25	%RSD	0.60
100% Sample	20	19.93	99.65	Mean	99.91
	20	20.06	100.3	SD	0.3403
	20	19.96	99.80	%RSD	0.34
120% Sample	24	23.98	99.91	Mean	100.17
	24	24.08	100.33	SD	0.2318
	24	24.07	100.29	%RSD	0.23

LINEARITY

The linearity of an analytical procedure is to elicit the test results are directly proportional to concentration. This is well understood by plotting a graph with peak area vs concentration.

Carried out the linearity studies for Pioglitazone hydrochloride each at different concentration levels of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml.

Acceptance criteria: Correlation coefficient should be not less than 0.9990. % of RSD for Level 1 and Level should be not more than 2.0 %.

Observation: The correlation coefficient was found to be 0.999 for Pioglitazone Hcl. From the above study it was established that the linearity of test method is from 10 µg/ml to 50 µg/ml of target concentration shown in linearity plots Table and figure no.1.0.

Table 5: Data for linearity.

Linearity					
S.No	**Concentration (µg/ml)**	**Area**	**Average Area**	**Standard deviation**	**% RSD**
1	10	2738401 2737299 2737181	2737627	672.89	0.02
2	20	6196977 6192976 6199022	6196325	3075.28	0.05
3	30	8931174 8924201 8928508	8927961	3518.53	0.04
4	40	11904956 11888002 11884634	11892530	10891.63	0.09
5	50	14447080 14445446 14443492	14445339	1796.37	0.01
Correlation Coefficient			**0.999065**		

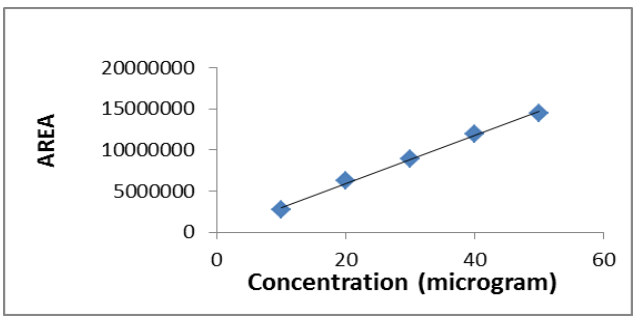

Figure 2.0: Linearity data for calibration curve.

RUGGEDDNESS: (Intermediate precision).

The United States pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

Preparation of Standard and Sample Solution

Standard and Sample solution were prepared as per test concentration level and injected 6 replicates and recorded the chromatograms. Calculated % RSD for Area for Pioglitazone Hydrochloride sample and analyzed 3 batches in the same sequence.

Preparation of Standard and Sample Solution

Standard solution is prepared as per test concentration level and injected 6 replicates and recorded the chromatograms. Calculated % RSD for Area for Pioglitazone Hydrochloride Analysed 3 batches in the same sequence. Details are given table 7.6(b) and fig.no:7.6.2.

Acceptance Criteria: The % RSD of Pioglitazone Hcl from the six sample preparations should be not more than 2.0 %.

***Observations*:** % RSD for Area in different days, analysts and systems are within the acceptance criteria were shown in table no: 6.0.

Table 6: Data for Summary of % RSD for Ruggedness.

Summary of Ruggedness by % RSD		
	Reference STD	**Sample**
Analyst-1	0.04	0.03
Analyst-2	0.15	0.66

ROBUSTNESS

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the robustness of proposed analytical method.

a) Change in column temperature
b) Change in flow rate
c) Change in column

a) Effect of Variation of temperature

The column oven temperature as per proposed analytical method is 25°C. Change in column oven temperature by 5°C. The effects due to change column oven temperature on the system suitability parameters are compared. Results are tabulated in table no.7.7(a) and 7.7(b).

***Acceptance criteria*:** The % RSD of Pioglitazone Hcl standard and sample solutions should be not more than 2.0 for variation in temperature.

Observation: Increase in temperature resulted in early elution. Decrease in temperature resulted in late elution. The resulted % RSD of Pioglitazone Hcl within the limit as shown in table.no:7.

b) Effect of variation of flow rate

Mobile phase flow rate as per proposed analytical method is 0.7 ml/min. Change in flow rate was at 0.5 and 1 ml/min. The effect due to change in flow rate on the system suitability parameters are compared. Results are tabulated in table no.7.

Acceptance Criteria: % RSD should not be more than 2.0%.

Observation: The % RSD for Pioglitazone Hcl found to be within the limits as shown in the table no7.

c) Effect of change in Column.

Standard and Sample solution is prepared as per test concentration level and injected 6 replicates.

Calculated % RSD for Area of Pioglitazone Hydrochloride, Analyzed 3 batches in the same sequence.

Acceptance Criteria: % RSD should not be more than 2.0% for Area.

Observation: The % RSD for Pioglitazone found to be within the limits as shown in the table no7.

Table 7: Summary data for % RSD of Robustness.

Summary of Robustness by % RSD		
Parameter	**Ref STD**	**Sample**

20°C	0.18	0.24
30°C	0.35	0.06
0.5ml/min	0.11	0.04
1.0ml/min	0.70	0.08
Column-1	0.05	0.15
Column-2	0.15	0.11

LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)

Prepare solutions of serial dilutions of concentration 0.2 µg/ml, 0.4 µg/ml, 1 µg/ml, 2 µg/ml,5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, 30 µg/ml.

Procedure

- Carry out the LOD & LOQ studies by injecting twice with the individual preparation of solutions.
- LOD & LOQ is obtained from the slope method.

SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tools of analysis. The estimation of Pioglitazone hydrochloride was done by Reverse Phase HPLC. The mobile phase used consists of Buffer containing Ammonium acetate and mobile phase ratio of Ammonium acetate: Acetonitrile: Glacial acetic acid. A C18 column containing Octadecyl silane (ODS) chemically bonded to porous silica particles (150 × 4.6mm, 5µ particle size) was used as the stationary phase. The detection was carried out using UV detector set at 269nm. The solutions are chromatographed at a constant flow rate of 0.7 ml/ min. The retention time for Pioglitazone hydrochloride was around 8.828 min. The quantitative estimation was carried out on the tablet using RP HPLC. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2.0%, indicating the accuracy and precision of the method. The percentage recoveries vary from 99.0 – 101.0% for Pioglitazone hydrochloride. The results obtained on the validation parameters met the ICH and USP requirements. It is inferred that the method was found to be simple, specific, precise and linear. The method was found to have suitable applications in routine laboratory analysis with high degree of accuracy and precision.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Azidus Laboratories, Chennai, India, for their valuable support in providing a literature survey and facilitating this research.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest related to this research.

REFERENCES

1. K.S Lakshmi, T. Rajesh, Shrinivas Sharma, "Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms". International Journal of Pharmacy and Pharmaceutical Sciences, Oct-Dec. 2009; 1(2).
2. Adukondalu D, P S Malathy, J Venkateshwar rao, Y Madhusudan Rao. Development and Validation of HPLC method for detection of pioglitazone hydrochloride in dosage forms. IJPBS, oct-dec 2011; 1(4): 474-478.
3. Sahoo P. K., R. Sharma, and S. C. Chaturvedi. Simultaneous Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride by RP-HPLC Method from Combined Tablet Dosage Form Indian J Pharm Sci, 2008 May-Jun; 70(3): 383–386.
4. Anand Prem D.C., K.L. Senthil kumar, B. Senthil kumar, M. Saravana kumar, R.Thirumurthy. A New RP-HPLC Method Development and Validation for Simultaneous estimation of Telmisartan and Pioglitazone in Pharmaceutical Dosage Form. International Journal of Chem Tech Research, Jan-Mar 2011; 3(1): 448- 454.
5. Ramzia I. El-Bagary, Ehab F. Elkady, Bassam M. Ayoub. Liquid Chromatographic Methods for the Determination of Vildagliptin in the Presence of its Synthetic Intermediate and the Simultaneous Determination of Pioglitazone Hydrochloride and Metformin Hydrochloride. International Journal of Biomedical Science, Sep 15, 2001; 7(3): 201-208.
6. Rashmitha N., Hiriyanna S.G, C H. Sreenivasa Rao, K. Chandra Sekhar Reddy, M. Hari Kiran, Hemant Kumar Sharmaa, K.Mukkanti, A validated stability indicating HPLC method for the determination of impurities in pioglitazone hydrochloride. Der Pharma Chemica, 2010, 2(5): 426-433.
7. AMR Lotfy Saber, Determination of Pioglitazone Hydrochloride in Tablets by High-Performance Liquid Chromatography, Pak. J. Anal. Environ. Chem, 2008; 9(2): 118. 121.
8. Srinivasulu D., B.S.Sastry, G. Omprakash, Development and Validation of new RP-HPLC method for determination of pioglitazone HCl in pharmaceutical dosage forms Int J Chem Res, 2010; 1(1): 1820.
9. Ravikanth CH, A. Anil Kumar, V Uday Kiran, S Prashanth, B Madhu, Y Narsimha Reddy. Sensitive and Rapid HPLC Method for the Determination of Pioglitazone in Rat

Serum. International Journal of Pharmaceutical Sciences and Drug Research, 2011; 3(1): 38-41.

10. Jedlicka A, Klimes J, Grafnetterová T. Reversed-phase HPLC methods for purity test and assay of pioglitazone hydrochloride in tablets. Pharmazie. 2004 Mar; 59 (3):178-82.
11. Rashmitha N., Hariyanna S. G, C H Sreenivasa Rao, K. Chandra Sekhar Reddy, M. Hari Kiran, Hemant Kumar Sharma, K. Mukkanti. A Validates Stability indicating method for the Determination of Impurities in Pioglitazone Hydrochloride. De Pharma Chemica, 2010, 2(5): 426-433.
12. Pattana Sripalakit, Penporn Neamhom, Aurasorn Saraphanchotiwitthaya. High Performance Liquid chromatographic method for the determination of Pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study. Journal of chromatography B., 7 November 2006; 843(2): 164-169.
13. Inamdar HP and Mhaske AA. RP-HPLC method for simultaneous determination of Metformin Hydrochloride, Rosiglitazone and Sitagliptin – application to commercially available Drug Products. Int J Pharm Sci Res, 2012; 3(9): 3267-3276.