



# **Comparison of the Robustness of Spectrophotometric Method and High-Performance Liquid Chromatographic Method for the Determination of Pioglitazone in Solid Dosage Form**

**Sara Zahid<sup>1\*</sup>, Fatima Zahid<sup>2</sup>, Asma Ahmed<sup>1</sup> and Waqas Safir<sup>3</sup>**

<sup>1</sup>*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan.*

<sup>2</sup>*Faculty of Pharmacy, The University of Lahore-Islamabad, Pakistan.*

<sup>3</sup>*Department of Biochemistry, Hazara University Mansehra, Pakistan.*

## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author SZ designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors FZ and WS did the experimental work. All authors managed the literature searches and read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/JPRI/2021/v33i1431273

### Editor(s):

(1) Dr. Aurora Martínez Romero, Juarez University, Mexico.

### Reviewers:

(1) Elham Y. Hashem, Assuit University, Egypt.

(2) Magdarita Riwu, Universitas Nusa Cendana, Indonesia.

(3) Amina Mohsen Abass, Al-Nahrain University, Iraq.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/66035>

**Original Research Article**

**Received 05 January 2021**

**Accepted 10 March 2021**

**Published 20 March 2021**

## **ABSTRACT**

Pioglitazone is a drug that reduces the amount of glucose (sugar) in the blood. It is included in the class of anti-diabetic drugs called "thiazolidinedione" that are used in the treatment of type II diabetes. It attaches to the peroxisomes proliferated- activated receptor gamma (PPAR $\gamma$ ) on tissues throughout the body and causes the cells to become more sensitive to insulin. As a result, more glucose is removed from the blood. The aim of the study is more precisely to find out the better analytical method for the quantitative measurement of the content of pioglitazone in commercially available drugs using two analytical methods i.e Spectrophotometric method and HPLC method. The analytical method for the pioglitazone hydrochloride was developed by HPLC,

\*Corresponding author: E-mail: [sara.zahid@imbb.uol.edu.pk](mailto:sara.zahid@imbb.uol.edu.pk), [sarazahid61@yahoo.com](mailto:sarazahid61@yahoo.com);

and then validated the method according to compendial requirements. Pioglitazone in various Dowglit and Gliden tablets was determined by this developed method. Precision, Accuracy and stability of Pioglitazone was checked which came out to be 100.40% and 100.19% respectively. The analysis of pioglitazone hydrochloride in solid dosage form using the HPLC method shows the results are more sensitive, accurate, validated and economical and can be easily applied to raw materials and finished goods compared to the Spectrophotometer method.

**Keywords:** Pioglitazone; HPLC; spectrophotometer; proliferated; insulin; anti-diabetic drugs.

## 1. INTRODUCTION

Pioglitazone hydrochloride [5-(4-(2-(5-Ethylpyridin-2-yl) ethoxy) benzyl) thiazolidine-2,4-dione hydrochloride (C<sub>19</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>3</sub>S)] is a thiazolidinedione antidiabetic agent (Fig. 1) which has its pharmacological action in the management of type II diabetes mellitus (Non-insulin dependent diabetes mellitus or adult-onset diabetes).

Antidiabetic mechanism of the drug depends upon the presence of insulin. Pioglitazone is responsible to decline insulin resistance in the periphery and in the liver resulting in glucose disposal which is insulin dependent and reduces hepatic glucose output. In contrast to sulfonylureas, Pioglitazone is not an insulin secretagogue. It is a potent agonist for peroxisome proliferator-activated receptor-gamma (PPAR $\gamma$ ) [2,3]. Peroxisome proliferator-activated receptor-gamma are located in liver, skeletal muscles and adipose tissues where transport of glucose is insulin dependent. Stimulation of PPAR $\gamma$  nuclear receptors modulates the transcription factors of many insulin responsive genes involved in glucose control and lipid metabolism. In diabetic animal model [4]. It has been found that pioglitazone decreases the clinical manifestation of Type II diabetes included hyperglycemia, hyperinsulinemia, and hypertriglyceridemia.

Responsiveness of insulin-dependent tissues increased due to metabolic changes produced by pioglitazone. As pioglitazone increases the response of circulating insulin by reducing insulin resistance, it does not decrease blood sugar level in animal models that are deficient in endogenous insulin [5,6]. Pioglitazone is extensively metabolized by oxidation and hydroxylation; the metabolites also partly convert to glucuronide or sulfate conjugates. Many metabolites including M-II and M-IV (hydroxyl derivatives of pioglitazone) and M-II (Keto derivative of pioglitazone) are found to be active pharmacologically in animal model of Diabetes

type-II. In Vitro data describe that multiple isoforms of CYP are involved in biotransformation of pioglitazone. CYP2C8 is involved majorly in the metabolism of Pioglitazone.

Approximately 15% to 30% of pioglitazone is recovered in urine after oral administration. Elimination of drug through renal route is negligible, and drug primarily excreted in the form of conjugates produced during Phase II reactions. It is assumed that major proportion of oral dose is excreted through bile salts and faeces [7,8]. The mean serum half-life of pioglitazone and total pioglitazone ranges from 3 to 7 hours and 16 to 24 hours, respectively. Pioglitazone has an apparent clearance ranges between 5 to 7 L/hour. Pioglitazone is available in various strengths including 15 mg, 30 mg and 45 mg tablets. The recommended dosing schedule of pioglitazone is once a day; it may also be initiated in combination of insulin at 15 mg or 30 mg once daily. The current dose of insulin can be continued with pioglitazone treatment [9,10]. In such patients who are receiving combination of insulin and pioglitazone, insulin dose can be reduced upto 25%. [11,12]. Further adjustment based upon glucose level. Various analytical techniques characterize assay method of drugs. Sample distillation is helpful in determination of alcohol contents of glanicals or other substances being volatile in steam including methanol, Thymol and even certain alkaloids such as ephedrine. Dessicator or heat oven has used for determination of moisture contents though most specific and convenient being Karl-Fisher [13,14]. Chromatography can be employed for separation of pharmaceuticals. Spectrophotometer can be considered an essential analytical tool. Which incorporate features such as microprocessor control and diode array detector. Fluorometer measures the fluorescence that may be present in the sample such as riboflavin or may be developed into sample as thiamine hydrochloride [15,16].

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

Dowglit and Gliden tablets (15 mg) containing Pioglitazone hydrochloride was purchased from Martin Dow Pharmaceuticals (PVT)Ltd and Reko Pharmcal (PVT) Ltd respectively. Ammonium acetate, Acetonitrile, Tetrahydrofolate (THF) of HPC grade and glacial acetic acid were purchased from Merck (Damstadt, Germany).

### 2.2 Instrumentation

The HPLC system consisted of a 515 pump, 710 plus Autosampler, and a variable 480 UV Detector. The data processing system was a multi-channel Chrome and Spec software for chromatography [17].Set up the instrument according to standard conditions. The blank was run. The following suitability of system criteria met prior to the injection of the sample preparation. Six repeat injection of standard preparation were made. %age relative standard deviation of five injections should not be greater than 2.0%. Performed three samples preparation injections for generic product and assay percentage was calculated.

### 2.3 Chromatographic Conditions

1.54 g of sodium acetate was measured accurately and then dissolved in 1000 ml of water. pH of buffer was adjusted with glacial acetic acid. 550 ml of this buffer was taken and made a volume of 1000 ml by using 450 ml of Acetonitrile (ACN). This mixture was filtered through Whatman filter paper No. 42 with pore size 45 micrometre and used as mobile phase. The column used was 5 $\mu$ m (250 x 4.6 mm). The optical density was set at 269 nm.

### 2.4 Preparation of Standard Solutions

Stock solution of Pioglitazone HCl was prepared by dissolving 11.5 mg of accurately weighed Pioglitazone HCl (100%) into 10 ml Tetrahydrofolate (THF) to dissolve and made a volume of 50 ml by using mobile phase. Solution was filtered through Whatman filter paper No. 42 [18]. Calibration curve samples were prepared from the stock solution to get six standard concentrations i-e 0.1, 0.2, 0.3, 0.5 1.0 and 2.0 mg/ml.

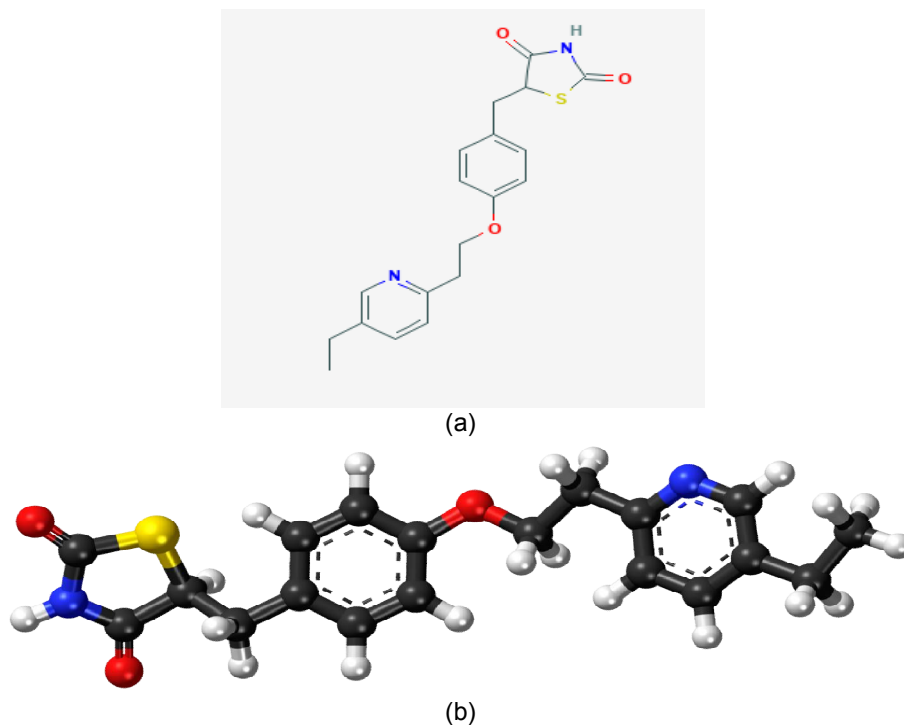


Fig. 1. Pioglitazone hydrochloride [1] (a) Molecular structure (b) Ball and stick model

## 2.5 Sample Preparation

20 tablets were taken and their average weight was calculated. The tablets were crushed to fine powder. Dose equivalent to 59 mg (active ingredient) was transferred to a 250 ml volumetric flask, and dissolved in 10 ml of tetrahydrofolate (THF) and then the volume of solution was made up to 100 ml by using mobile phase. 10 ml of this solution was taken and made up the volume 25 ml by using mobile phase [19].

## 3. RESULTS

### 3.1 Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of homogeneous sample. Six injections of sample solution (concentration 0.2 mg/ml) gave the results in the range of (98.52% - 101.05%) which are shown in the Table 1. (Limit = SD NMT 2.0).

### 3.2 Accuracy

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range. Weight of the Pioglitazone HCL (100% or 0.2 mg/ml) was added to the amount of placebo mixture equivalent to the amount of placebo present in the sample solution preparation. Three solutions were prepared and spiked and results were calculated and given in Table 2.

### 3.3 Stability

Stability of the solution is also tested to ensure the validity of analytical procedure. Injections of

the sample solutions at room temperature over the period of 5hrs showed no significant difference in peak area of Pioglitazone hydrochloride over this time period, results are given in Table 3.

### 3.4 Linearity

The linearity of an analytical procedure is its ability to elicit results directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within the given range. Solutions of Pioglitazone hydrochloride were produced in mobile phase having concentrations in the range of active/inactive 0.0008 – 0.24 mg/ml (0.40 -120%) of the theoretical concentration in the sample preparation 90.2 mg/ml). This method shows the linear response with these concentrations shown in Table 4.

### 3.5 Specificity

Specificity is the ability to assess the analyte in the presence of components that may be expected to the present, such as impurities, degradation products, and matrix components. A weight of placebo mixture equivalent to the amount present in the sample solution preparation was taken through HPLC procedure. No interference was observed in the chromatogram. Hence specificity is confirmed.

### 3.6 Quantitation Limit

The quantitation limit is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk substances and degradation products in finished pharmaceuticals. The quantitation limit is expressed as the concentration of analyte. Detection limit is the slowest amount of analyte in a sample that can be determined

**Table 1. The intraday precision of the HPLC method**

Sr. No.	Peak Area	% age with Avg.
1.1	1557075	98.76%
1.2	1553253	98.52%
1.3	1573400	99.80%
1.4	1589714	100.83%
1.5	1593138	101.05%
1.6	1592916	101.04%
Average	1576583	100.00%
SD		0.011

**Table 2. Accuracy of the HPLC method**

Sr. No.	Conc. mg/ml	%age of soln	Peak Area	Average	Recovery	% age with actual
2.1	0.15	75%	1185094	1190382	75.70%	100.90%
2.2			1180201			
2.3			1205851			
2.4			1568659			
2.5	0.200	100%	1561469	1573181	100.00%	100.00%
2.6			1589415			
2.7			1857338			
2.8	0.240	120%	1866273	1872496	119.03%	99.19%
2.9			1893877			
				Average		100.04%
				SD		0.007

**Table 3. Stability of the HPLC method**

Sr. No.	Conc. mg/ml	%age of solution	Recovery	% age with actual
1	0.150	75%	75.70%	100.93%
2	0.200	100%	100.00%	100.00%
3	0.240	120%	119.03%	99.19%

**Table 4. Linearity of the HPLC method**

Sr. No.	Time after Preparation hours	Peak Area	% Age of Initial value
1	0	1557075	100.00
2	1	1589714	102.01
3	3	1611055	103.47
4	5	1624767	104.35
Average		1595653	102.46
SD			1.65

**Table 5. Specificity of the HPLC method**

Sr. No.	Conc. mg/ml	Peak Area	%age of theo. Conc	%age from area with theo. Conc	%age of Linearity
1	0.0008	12851	0.04	0.82	205.00
2	0.002	31519	1.00	2.00	200.00
3	0.02	152646	10.00	9.70	97.00
4	0.03	235560	15.00	14.97	99.80
5	0.046	366824	23.00	23.32	101.40
6	0.12	972441	60.00	61.81	103.01
7	0.15	1190382	75.00	75.70	100.93
8	0.2	1573181	100.00	100.00	100.00
9	0.24	1872496	120.00	119.03	99.19
				Average	101.29
				SD	1.79

**Table 6. Quantitation limit of HPLC method**

Sr. No.	Conc. mg/ml	Peak Area	
1	0.0008	12851	Detection Limit
2	0.002	31519	
3	0.02	152646	Quantitation
4	0.03	235560	
5	0.046	366824	
6	0.12	972441	
7	0.15	1190382	
8	0.2	1573181	
9	0.24	1872496	

**Table 7. Determination of %age of Pioglitazone by U.V/visible method**

Sr. No.	Peak Area	% Age with Avg.
1.1	1557075	98.76%
1.2	1553253	98.52%
1.3	1573400	99.80%
1.4	1589714	100.83%
1.5	1593138	101.05%
1.6	1592916	101.04%
Average	1576583	100.00%
SD		0.011

**Table 8. Determination of retention time of Pioglitazone by the HPLC method**

Injection no.	R. Time	Peak Area
1.1	9.391	1714720
1.2	9.385	1714689
1.3	9.365	1736419
1.4	9.372	1740640
1.5	9.366	1712772
1.6	9.365	1736419
Average		1729943

**Table 9. Analysis of Pioglitazone by spectrophotometric method**

Wavelength(nm)269	Absorbance
	0.5107
	0.5120
	0.5127
Average	0.5118
269	0.5042
	0.5045
	0.5027
Average	0.5038
269	0.5488
	0.5527
	0.5522
Average	0.5512

**Table 10. Analysis of Pioglitazone in standard drugs by HPLC and Ultraviolet-visible spectroscopy**

Sr. No	Product	Assay by HPLC	Assay by UV
1.	Dowglit Tablets	100.40%	98.44%
2.	Gliden Tablets	100.19%	100.98%

with acceptable precision and accuracy under the stated experimental conditions. The detection limit is a characteristic of limit tests. It is the lowest amount of analyte a sample that can be detected, but not necessarily quantities under the stated experimental conditions. Thus, limit test shows the amount of analyte is above or below a certain level. The detection limit is usually expressed as the concentration of analyte in the sample. Linearity curve shows that upto concentration of 0.02 mg/ml, curve is linear. So, pioglitazone HCl was quantified at this concentration i.e 0.02 mg/ml. Beyond this concentration we can detect the pioglitazone HCl at 0.0008 mg/ml (Table 6).

#### 4. DISCUSSION

The aim of the study was to determine a simple, cost effective HPLC method that could be conveniently used for the routine analysis of large number of samples usually obtained in pharmacokinetic studies. Only few HPLC methods are available for the quantification of Pioglitazone, therefore, the present work illustrates the robustness and ruggedness of HPLC analytical method as compared to spectrophotometric method for the analysis of Pioglitazone hydrochloride in solid dosage form. Before starting the work, a thorough search of literature was carried out using various search engines to study methods available for the quantification of pioglitazone in biological samples. To the best of our knowledge, the HPLC methods reported to-date for pioglitazone are either difficult or requires larger samples after extraction. The liquid chromatography–tandem mass spectrometry (LC-MS/MS), liquid chromatography–electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) methods, although sensitive enough but are costly and not suitable for routine analysis. This analytical method for the pioglitazone hydrochloride was developed by HPLC, and then validated the method according to compendia requirements. Pioglitazone in various pharmaceutical products was determined by this developed method. Different concentrations of standard solution were prepared and run on HPLC and also prepared the sample solution and compare its chromatograms with standard solutions. Assay of these products was in the official limits. And finally, when the results obtained by this method were crosschecked with U.V. method. The precision of the developed method was checked by intra-day repeatability of responses after replicate injections of sample solution with

percentage of average as 98.6%, 98.52%, 99.0%, 100.83%, 101.05% and 101.04% showing high precision rate (Table 1). The specificity of the developed method was observed by analysing placebo (including all the ingredients of the formulation except the analyte), standard solution and market formulation containing pioglitazone. No peaks were observed close to the wavelength of detection and hence proved high specificity of the method. (Table 4). The accuracy was evaluated at three different successive concentrations using the proposed developed method and the value as expressed as percentage of recovery (R%) between the average concentrations. The average percentage of recovery was found to be 100.90%, 100.0% and 99.19% for 0.15, 0.200 and 0.240 mg/ml respectively (Table 2). The quantitation of the active drugs was determined for two commercial formulations (Dowglit and Gliden tablets) and the potency of pioglitazone by developed HPLC method was found to be 100.40% and 100.9% respectively as compared to the UV/visible spectrophotometric method (98.44% and 100.988% respectively). Therefore, it was found that HPLC method is a precise and accurate, highly sensitive, rapid and effective, mixture of substance can be analysed without any interference and gave results with less errors.

#### 5. CONCLUSION

The objective of better quantitative determination of pioglitazone in solid dosage form was obtained by more sensitive high performance liquid chromatographic method which was approved and validated by the guidelines of FDA, ICH and USP. In spite of the good separation and resolution of the chromatographic peaks, it is concluded that HPLC is more sensitive, rapid and effective method for the determination of Pioglitazone hydrochloride in raw material and finished pharmaceutical products. Although it requires high operating techniques but it is more reliable for both qualitative and quantitative analysis of pioglitazone hydrochloride in raw material and solid dosage forms. Also, its accuracy has proved with comparative study with U.V. Visible spectroscopy.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- American Society of Health-System Pharmacists. Pioglitazone Hydrochloride Monograph for Professionals. 2020.
- Abdel Ghany MF, Ayad MF, Tados MM. Enhanced LC-MS/MS analysis of alogliptin and pioglitazone in human plasma: Applied to a preliminary pharmacokinetic study. *J. Chromatogr. B.* 2017;1058:93-101.
- Abushammala I. The Effect of Pioglitazone on Pharmacokinetics of Carbamazepine in Healthy Rabbits. *Saudi Pharm. J.* 2015; 23(2):177-181.
- Brayfield A. Martindale: The complete drug reference, Pharmaceutical Press. 2014;22-26.
- Cheng TY, Lee WS, Huang HC, Lee FY, Chang CC, Lin HC, et al. The effects of pioglitazone in cirrhotic rats with hepatopulmonary syndrome. *J. Chin. Med. Assoc.* 2017;80(11):683-689.
- Chinnalalaiah R, Pigili R, Avanapu SR. Liquid chromatography and tandem mass spectrometry method for quantitative determination of pioglitazone and its metabolite 5-hydroxy pioglitazone in human plasma. *Ann. Pharm. Fr.* 2017;75(2):105-111.
- Dey S, Subhasis Patro S, Suresh Babu N, Murthy PN, Panda SK. Development and validation of a stability-indicating RP-HPLC method for estimation of atazanavir sulfate in bulk. *J. Pharm. Anal.* 2017;7(2):134-140.
- Food and drug administration. FDA guidance for industry. Bioanalytical method validation. US department of health and human services, food and drug administration, Center for Drug Evaluation and Research (CDER). Food and Drug Administration. 2017;21-26.
- Ghoreishia SM, Rajaianb H, Sheykhzadec M, Alikhania M, Rahmania HR, Hajipour AR. Pharmacokinetics of pioglitazone, a thiazolidinedione derivative, in male Naeini (Iranian fat-tailed) sheep. *J. Appl. Anim. Res.* 2012; 40(3):208-214.
- Hiroi S, Matsuno K, Hirayama M, Hayakawa T, Yoshioka N, Kawakami K. Bioequivalence evaluation of pioglitazone orally disintegrating tablet formulation. *Diabetes Manage.* 2012;2:3-11.
- Rezk MR, Riad SM, Mahmoud GY, Aleem A. Simultaneous determination of pioglitazone and glimepiride in their pharmaceutical formulations. *J. Appl. Pharm. Sci.* 2012;2:177-184.
- Macha S, Mattheus M, Pinnetti S, Broedl UC, Woerle HJ. Pharmacokinetics of empagliflozin and pioglitazone after coadministration in healthy volunteers. *Clin. Ther.* 2015;37(7):1503-1516.
- Mujtaba A, Kohli K. Validated HPLC method for the pharmacokinetic study of oral extended-release cefpodoxime proxetil chitosan-alginate beads in rabbits. *Int. J. Biol. Macromol.* 2017;98:111-116.
- Ni XJ, Wang ZZ, Shang DW, Zhang M, Hu JQ, Qiu C, et al. Simultaneous determination of glimepiride and pioglitazone in human plasma by liquid chromatography-tandem mass spectrometry and its application to pharmacokinetic study. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2014; 960:247-252.
- Satheeshkumar N, Shantikumar S, Srinivas R. Pioglitazone: A review of analytical methods. *J. Pharm. Anal.* 2014;4(5):295-302.
- Sengupta P, Chatterjee B, Mandal UK, Gorain B, Pal TK. Development and validation of a high throughput LC-MS/MS method for simultaneous quantitation of pioglitazone and telmisartan in rat plasma and its application to a pharmacokinetic study. *J. Pharm. Anal.* 2017;7(6):381-387.
- Effat S, Hassan J, Shahrooz S. Development and validation of a simple



- and rapid HPLC method for determination of pioglitazone in human plasma and its application to pharmacokinetic study. Journal of Chromatographic studies. 2008; 46:809-812.
18. Srinivasulu D, Sastry BS, Omparkash G. Development and Validation of new RP-HPLC method for determination of Pioglitazone HCl in Pharmaceutical Dosage Form. International Journal of Chemistry Research. 2010;2(1):18-20.
19. United States Pharmacopoeia - National Formulary, USP 37 - NF 32. Pioglitazone hydrochloride tableFs. United States Pharmacopeial convention, Rockville, MD. 2014;4310-4311

---

© 2021 Zahid et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle4.com/review-history/66035>