EFFICACY AND SAFTEY OF PIOELITAZONE IN TYPE 2 DIABETES BY USING RP-HPLC METHOD

BONAGIRI SREEDEVI [*1], B. ANUSHA REDDY, SREEKANTH, B. ANUSHA, B. SREEJA REDDY, RAMU, B. HARITEJA

Department of Pharmaceutical Analysis and Quality Assurance[1], Teegala Ram Reddy college of pharmacy, Telangana, India

Abstract : The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Pioelitazone in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Symmetry C18, 250 mm x 4.6 mm and 5µm Column,Mobile Phase : Phosphate buffer(PH 4.0) :Methanol = 50:50,flow 1.0 ml/min with Injection Volume 20µl, at detection wavelength 255 nm, run time at 08 mins and Retention time was 2.689mins.The analytical method is valid for estimation of Pioglitazone over a range of 0-35 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Pioglitazone has been developed based on ICH Guidelines with bulk and dosage forms.

Key Words: Pioglitazone, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

I.INTRODUCTION

Pioglitazone is used for the treatment of diabetes mellitus type 2. Pioglitazone selectively stimulates nuclear receptor peroxisone proliferator-activated receptor gamma (PPAR-gamma). It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the lipidic, muscular tissues and in the liver

5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione.

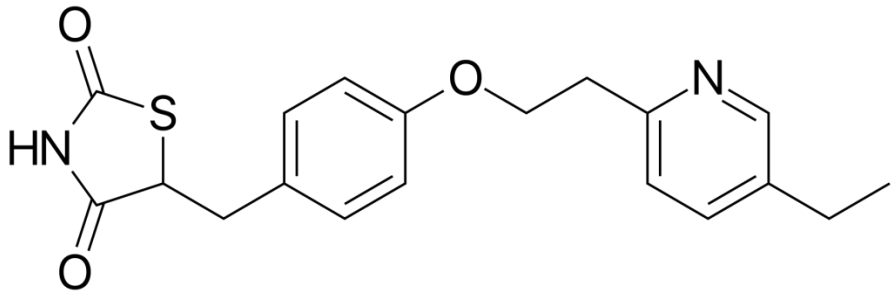


Fig-1: Structure of Pioglitazone

II.EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Pioglitazone were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (T60-LAB INDIA), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Symmetry C18 Column, 250 mm x 4.6 mm and 5µm (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

10 mg of Pioglitazone standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 100ppm concentration. Then, the final concentration was made by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration. It is scanned in the UV spectrum in the range

of 200 to 400nm.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in methanol diluting with the same solvent. (After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Pioglitazone solution we observed the maxima at 255 nm.

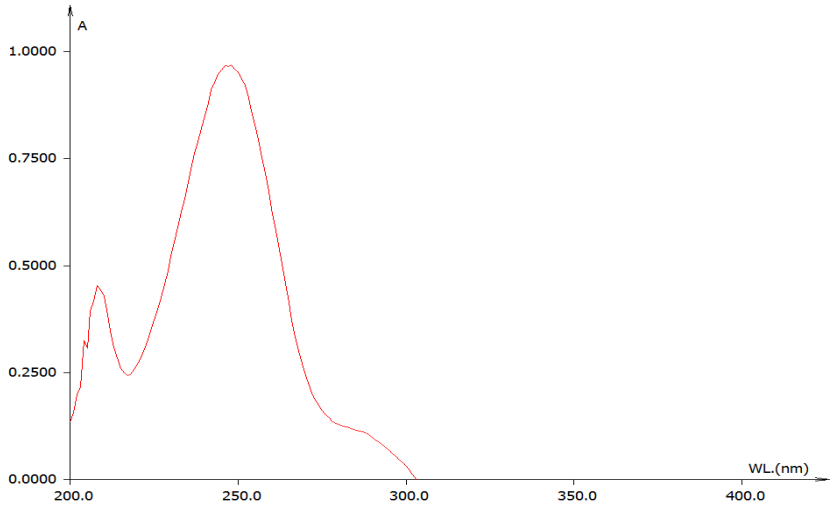


Fig-2: UV Spectrum for Pioglitazone

2.5 Method Development

2.5.1 Preparation of Mobile Phase:

The mobile phase used in this analysis containing of a mixture of Phosphate buffer:Methanol in a ratio of 50:50v/v was prepared in the volume of 1000ml in which 500ml of Methanol was mixed with 500ml of Water.

2.5.2 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimized Chromatographic Conditions

Column	Symmetry C18 Column, 250 mm x 4.6 mm and 5µm
Mobile phase	Phosphate buffer(PH 4.0) :Methanol = 50:50
Wavelength	255 nm
Flow rate	1.0 ml/ min.
Sampling System	Automatic
Injection Volume	20µl
Run Time/ Stop Time	08minutes
Concentration of Sample	10ppm

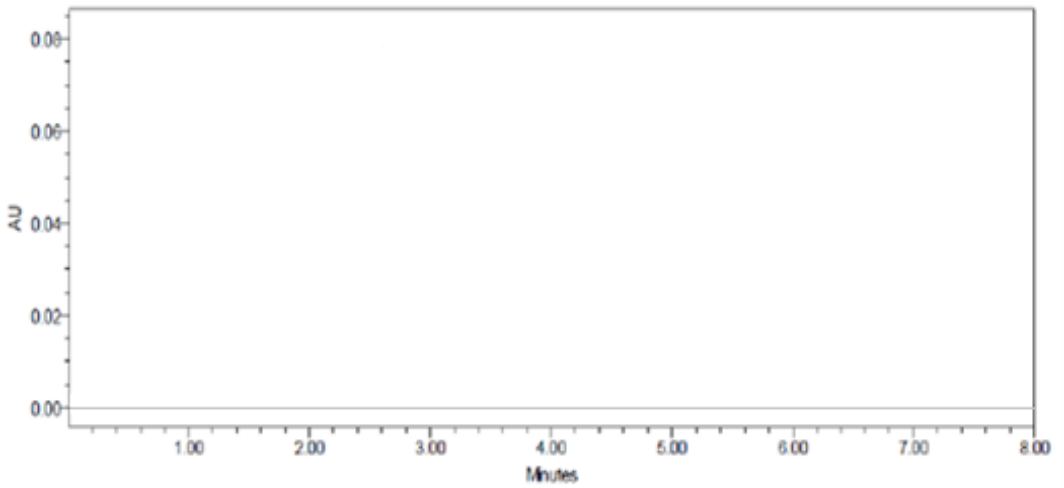
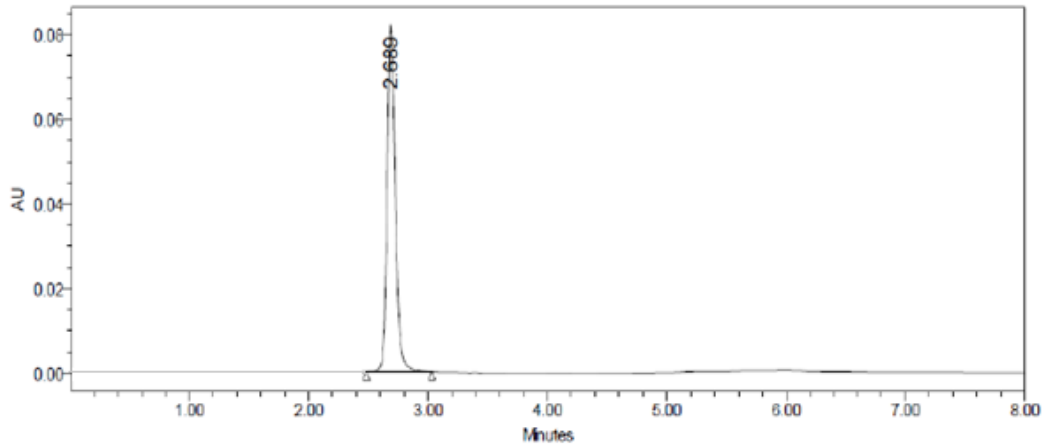


Fig-3: Chromatogram for Blank Preparation



Fig–4: Chromatogram of Pioglitazone in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 6-14 µg/ml for Pioglitazone. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

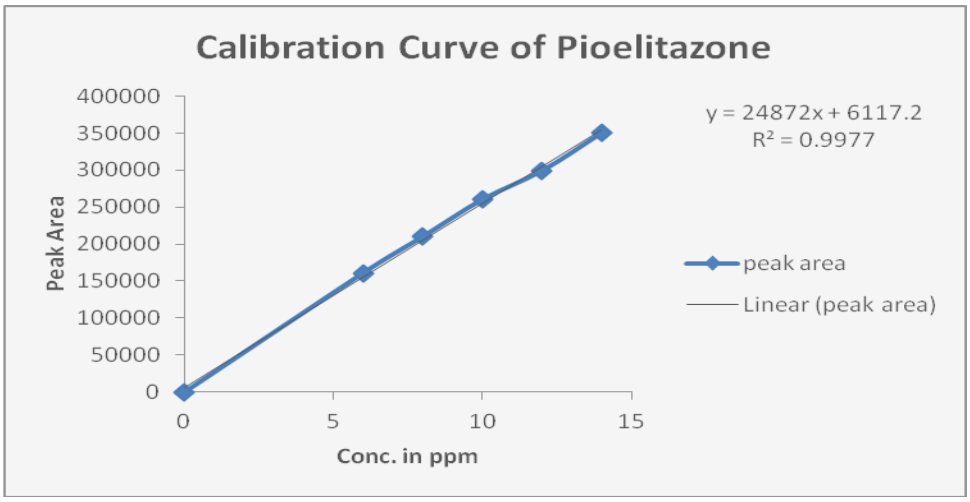


Fig-5: Calibration Curve of Pioglitazone (API)

Table-2: Concentration of Pioglitazone

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	161005
3	8	210083
4	10	260265
5	12	298963
6	14	349993

2.6.2. Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Pioglitazone were taken and added to the pre-analyzed formulation of concentration 20µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table-6.1: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis	
	Amount Added	Amount Found				
S_1 : 80 %	8	7.938525	203564	99.23157	Mean = 99.55389333 S.D. = 0.891664568 % R.S.D.= 0.8956606017	%Mean Recovery = 100.07
S_2 : 80 %	8	8.04495	206211	100.5619		
S_3 : 80 %	8	7.909456	202841	98.86821		
S_4 : 100 %	10	10.08182	256872	100.8182	Mean = 100.51666 S.D. = 1.199579441 % R.S.D. = 1.1934135505	
S_5 : 100 %	10	9.919508	252835	99.19508		
S_6 : 100 %	10	10.15367	258659	101.5367		
S_7 : 120 %	12	11.77356	298949	98.11301	Mean = 99.39210667 S.D. = 1.117473625 % R.S.D. = 1.1243082197	
S_8 : 120 %	12	12.02147	107546	100.2623		
S_9 : 120 %	12	11.98613	106574	99.35581		

2.6.3. Precision:

2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Pioglitazone (API). The percent relative standard deviation was calculated for Pioglitazone are presented in the Table-4.

Table-4: Repeatability Readings of Pioglitazone

HPLC Injection Replicates of Pioglitazone	Peak Area
Replicate – 1	221707

Replicate – 2	220797
Replicate – 3	221508
Replicate – 4	221507
Replicate – 5	231872
Replicate – 6	221757
Average	**223191.3333**
Standard Deviation	**4266.566613**
% RSD	**1.91161**

2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Pioglitazone revealed that the proposed method is precise.

Table-5: Results of Intra-Assay & inter-assay

Conc. Of Pioglitazone (API) (µg/ml)	Observed Conc. Of Pioglitazone (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.08	0.96	8.03	0.97
10	10.04	0.40	10.03	0.42
12	12.97	0.33	12.95	0.14

2.6.4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-6, % RSD < 2%) the developed RP-HPLC method for the analysis of Pioglitazone (API).

Table-6: Results of Method Robustness Test

Change in parameter	**% RSD**
Flow (1.1 ml/min)	0.02
Flow (0.9 ml/min)	0.08
More Organic (70+5)	0.04
Less Organic (70-5)	0.16
Wavelength of Detection (252 nm)	0.05
Wavelength of detection (250 nm)	0.07

2.6.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 0.33(SD/S).
L.O.Q. = 0.10(SD/S)
Where, SD = Standard deviation of the response
S = Slope of the calibration curve

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27 µg/ml respectively.

2.6.6. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-7.

Table-7: Data of System Suitability Parameter

S. No	Parameter	Pioglitazone
1	Retention time	2.689
2	Tailing factor	T ≤ 2
3	Theoretical plate	N > 2000

2.6.7 Estimation of Pioglitazone in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with mobile phase. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

Table-8: Assay of Pioglitazone Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Pioelitazone Tablets(Care Formulation Lab PVT LTD)	15	14.85 (±0.06)	99.37 (±0.47)

Result& Discussion: The %Purity of Pioelitazone Tablets containing Pioelitazone was found to be 99.37 %.

III. RESULTS

The optimized The chromatographic conditions were performed on Symmetry C18, 250 mm x 4.6 mm and 5µm Column, Mobile Phase: Phosphate buffer (PH 4.0): Methanol = 50:50, flow 1.0 ml/min with Injection Volume 20µl, at detection wavelength 255 nm, run time at 08 mins and Retention time was 2.689mins.

The results obtained in method validation were:

Linearity & Range: We observed that the calibration curve showed good linearity in the range of 6-14 µg/ml, for Pioelitazone (API) with correlation coefficient (R2) of 0.999 (Fig-6.1). A typical calibration curve has the regression equation of y = 24872x + 6117 for Pioelitazone.

Accuracy: From the Accuracy Method, we observed that the mean %Recovery of the drug are 99.8 which is within the range of 98-102% and %RSD is within the range <2 i.e. 0.8956606017%, 1.1934135505% and 1.1243082197% respectively.

Repeatability: From the Precision method, we observed that the %RSD of the AUC is 1.91161 and RT is 0.585867359 which are within the acceptable range as per ICH guidelines.

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27 µg/ml respectively.

Assay: The %Purity of Pioelitazone Tablets containing Pioelitazone was found to be 99.37 %.

IV. DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Dapagliflozin, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Madhukar Akkala, et al Separation was achieved with an Symmetry - Extend - C18 HPLC column 150mm in length and having an internal diameter of 4.6mm. A mobile phase comprising 0.01M Buffer: Methanol in the volume ratio of (40:60) was developed. The detection was carried out using a UV-detector set at a wavelength of 240nm. Validation experiments were performed to demonstrate System suitability, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 1-200µg/ml and get the correlation Regration (r2) 0.999, showed good recoveries (99.3 - 103.2%). The method can be used for quality control assay of Pioglitazone Hydrochloride.

V. CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Pioelitazone API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Pioglitazone indicated that the developed method is specific for the estimation of Pioglitazone. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

REFERANCE

[1] https://www.longdom.org/scholarly/pharmaceutical-analysis-journals-articles-ppts-list-1953.html
[2] Olander, D.P., Instrumental Methods of Analysis, SixthEdition (Willard, Hobart H.; Merritt, Lynne L.; Dean, John A.; Settle, Frank A., Jr.). Journal of Chemical Education, 1984. 61(8): p. A222.
[3] Lipka, E. and C. Vaccher, Quantitative analysis of drugs in biological matrices by HPLC hyphenated to fluorescence detection. Bioanalysis, 2015. 7(6): p. 743-762.
[4] Choudhury, H., et al., Development and validation of RP-HPLC method: scope of application in the determination of oil solubility of paclitaxel. J ChromatogrSci, 2014. 52(1): p. 68-74.
[5] Md Sabir, A., M. Moloy, and P. S Bhasin, HPLC METHOD DEVELOPMENT AND VALIDATION: A REVIEW. Vol. 4. 2015. 39-46.
[6] Malherbe, C.J., D. de Beer, and E. Joubert, Development of on-line high performance liquid chromatography (HPLC)-biochemical detection methods as tools in the identification of bioactives. Int J Mol Sci, 2012. 13(3): p. 3101-33.
a. International Journal of Trend in Scientific Research and Development (IJTSRD) ISSN: 2456-6470 @ IJTSRD |
[7] https://www.knauer.net/en/Systems-Solutions/Analytical-HPLC-UHPLC/HPLC-Basics---principles-and-parameters
[8] Hopmann, E., W. Arlt, and M. Minceva, Solvent system selection in counter-current chromatography using conductor-like screening model for real solvents. J Chromatogr A, 2011. 1218(2): p. 242-50.
[9] Xiang, Y., Y. Liu, and M.L. Lee, Ultrahigh pressure liquid chromatography using elevated temperature. J Chromatogr A, 2006. 1104(1-2): p. 198-202.
[10] Martin, M. and G. Guiochon, Effects of high pressure in liquid chromatography. J Chromatogr A, 2005. 1090(1-2): p. 16-38.
[11] Madhukar Akkala., et al, 2011,Rapid and sensitive RP-HPLC analytical method development and validation of Pioglitazone Hydrochloride, Scholars Research Library Der Pharmacia Lettre, 2011, 3(3): 128-132