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RP-HPLC Method Development and Validation for Pioglitazone in Bulk and Marketed Formulation

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ABSTRACT

The work describes a precise, accurate and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for development and validation of Pioglitazone in tablet dosage form on Lachrome Liquid chromatographic system having PDA-20 A UV/VIS Detector using stationary phase C18 column (300 mm × 3.9 mm, 5 µm, particle size) and acetonitrile:phosphate buffer, (50:50 v/v) as mobile phase at flow rate of 1.00 ml/min and the detection wavelength was 267 nm. The retention time for Pioglitazone was found to be 8.08 min. The method was validated for precision, accuracy, linearity range, robustness, system stability, as per ICH guidelines Q2(R1).

Keywords: Pioglitazone, RP-HPLC, PDA detector, C18 column

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 ant-diabetic 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. [8-24] The current study has been undertaken to develop RP-HPLC method for the determination of Pioglitazone in bulk and pharmaceutical formulation.

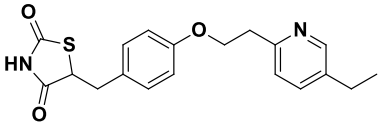

Figure 1: Pioglitazone

EXPRIMENTAL

Materials and Method

Pioglitazone was obtained as a gift sample from Dr. Reddy's Laboratories.Pioglitazone tablets were procured from the local market. HPLC grade Acetonitrile, AR grade Diammonium hydrogen orthophosphate, Potassium dihydrogen orthophosphate, orthophosphoric acid and triethylamine were used for the analysis, distilled water was utilized from Merkmilipore.

Instrument and Column

The Lachrom 2200 system equipped with auto sampler, and Lachrom elite control as the operating software. The chromatographic separation was carried out on C18 column, 300x3.9mm, 5µm (Bondapack column).

Preparation of Mobile Phase

Mixture of buffer (1.15 gm of diammonium hydrogen orthophosphate, 1.36 gm of potassium dihydrogen orthophosphate, 1ml triethylamine)and acetonitrile in the ratio of (50:50v/v)was filter, degased and used for analysis.

Preparation of Buffer

1.15gm of diammonium hydrogen orthophosphate, 1.36 gm of potassium dihydrogen orthophosphate and 1ml triethylamine was dissolved in 1000 ml water,pH was adjusted to 5.0 with orthophosphoric acid.

Preparation of Standard stock solution

30mg of Pioglitazone Hydrochloride was placed in 100ml volumetric flask and 10ml of dimethyl formamide was transferred to the volumetric flask and sonicated for 15 min. and the solution was made up to the mark using mobile phase. Then 2ml of the above solution was transferred to 25ml volumetric flask and the volume was made upto the mark using mobile phase.

Preparation of Sample Solution

Weighed and powder 20 tablets. Transferred equivalent weight 30mg of pioglitazone to a 100 ml volumetric flask. Add about 10 ml dimethyl formamide and sonicate for 5 min. Add 70 ml of mobile phase and sonicate for 10 min. Cool and dilute up to the mark with mobile phase . Filtered through whatman filter paper no.1. Transfer 2 ml of filtrate to 25 ml volumetric flask and dilute with mobile phase.

Chromatographic condition

The optimized chromatographic conditions and the optimized chromatogram for the newly developed method have been represented in Table 1 and Figure 2 respectively.

Table 1: Optimized Chromatographic Conditions.

Mobile Phase	Filtered and degassed Buffer:Acetonitrile (50:50%v/v)
Stationary Phase	Bondapack C18, 300×3.9 mm 5µm
Detection wavelength	267nm
Run Time	10 min
Flow rate	1.0 ml/min
Injection Volume	20 µL
Column Temperature	25° C
Retention Time	8.08 min

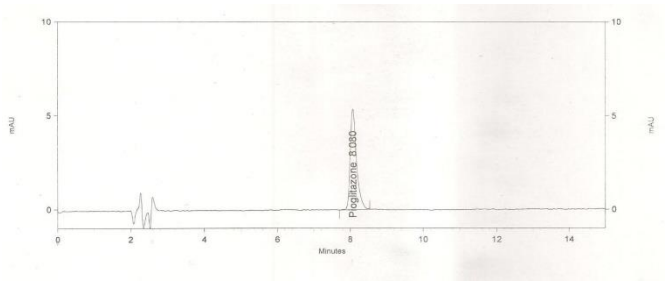

Figure 2: Optimized Chromatogram of Pioglitazone.

Method Validation

The method was validated as per ICH guidelines Q2(R1), in terms of System suitability, Linearity, Precision, Accuracy, and Robustness [25-30].

System Suitability Tests for Validation

System suitability tests is performed to ensure system performance before and duringthe analysis which demonstrates that the system is operating properly and ready todeliver results with acceptable accuracy and precision. Five replicate injections of a single standard solution were made on to a RP-HPLC system and the area of the pioglitazone peak was determined. USP Tailing for pioglitazone was recorded. The relative standard deviation of the peak area was calculated. The other parameters considered for system suitability were USP plate count for pioglitazone. The limit set and the values are reported given in Table 2.

Linearity

The linearity of analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample [28]. 7 different solutions of pioglitazone ranging from 11.82 ppm to 35.46 ppm were prepared and analysed. Concentration was plotted on X-axis and area on Y-axis. Correlation coefficient and the equation of line were calculated. The data obtained and the graph of Linearity has been represented in Table 3 and Figure 3 respectively.

Range

Five replicates of each linearity level, 50% level (lower level) and 150% (upperlevel) was injected and %RSD for retention time and area were determined. The data is summarized in Table 4.

Precision

System Precision

Repeatability of pioglitazone standard in assay method.5 replicates of standard preparation were injected as per method parameters and the %RSD for the peak area and retention time was determined. The data has been summarized in Table 5.

Method Precision

The assay percentage for each test preparation and the mean assay of six test preparation and %RSD for the same was calculated and the data is summarized in Table 6.

Intermediate Precision

The intermediate precision was performed on different days, equipment and analyst. The data for interday precision has been given in Table 7, the data for intraday day has been given in Tables 8 & 9 exhibits the results of one way ANOVA.
Equipment used: HPLC
Make: Shimadzu
Model: Class VP
Column: C18, 300*3.9 mm, 5 µm (Bondapack is suitable)

Reproducibility

Blank preparation: Mobile phase is used as blank preparation.

Placebo preparation

170.4 mg of placebo is weighed and transferred to a 100 ml volumetric flask. 10 ml of dimethyl formamide was added and sonicated for about 5 minutes. 70ml of mobile phase was added and sonicated for 10 min. Cooled and diluted upto the mark with mobile phase followed by transfer of 2.0 ml of filtrate to a 25 ml volumetric flask, diluted to volume with mobile phase.

Sample preparation
202.5 mg of sample (equivalent to 30 mg of pioglitazone) is weighed and transferred to a 100 ml volumetric flask. 10 ml of dimethyl formamide was added and sonicated for about 5 minutes. 70ml of mobile phase was added and sonicated for 10 min. Cooled and diluted upto the mark with mobile phase followed by transfer of 2.0 ml of filtrate to a 25 ml volumetric flask, diluted to volume with mobile phase. In continuation with the above experiment prepare and analyze six different independent samples as per method. The assay percentage for each test preparation and The mean assay of six test preparation and %RSD for the same was calculated. The data obtained is summarized in Table 10.

Accuracy

Known amount of the active ingredient at 3 levels each in triplicate, i.e. 3 x 80%, 3 x100% and 3 x 120% of the working concentration was spiked with placebo at 100 %level of 100 mg tablet samples were prepared in triplicate. Each sample was analyzedand calculated. The data for individual compound is summarized in Table 11.

Robustness

To demonstrate the robustness of the test method checked the method suitability by injecting test solution into RP-HPLC with slight variations in method parameters[31]. Standard solution and test solution were prepared as per method of analysis. Once blank and five replicate of standard injection and sample solution in duplicate were injected. The mean % assay for sample solution with slight variation in method parameter was calculated. Changes in chromatographic conditions were as follows:

1. Change in flow ± 10%
2. Change in organic phase ± 10%
3. Change in pH ± 0.2

The values for Robustness has been represented in Tables 12-17, and the chromatogram of blank solution has been represented in Figure 3.

RESULTS AND DISCUSSION

To develop RP-HPLC method, several mobile phase and mobile phase compositions were tried. A satisfactorily separate and good peak symmetry was obtained with Bondapack 300*3.9mm 5µm C18 column using mobile phase Acetonitrile:Buffer (50:50%v/v) and flow rate 1.0 ml per min. The detection was carried out 267 nm and the retention time was found to be 8.08 min.

System Suitability for validation

Five replicate injections of a single standard solution were made on to a RP-HPLC system and the area of the pioglitazone peak was determined. USP Tailing for pioglitazone was recorded. The relative standard deviation of the peak area was calculated. The other parameters considered for system suitability were USP plate count for pioglitazone. The limit set and the values obtained are in below in Table 2.

Table 2: The limit set and the values obtained for system suitability.

Parameter	Set limits	Obtained Values
% RSD of peak area for five replicate injections for pioglitazone in 45 mg standard	NMT 2.00%	0.62%
% RSD of retention time for five replicate injections for pioglitazone in 45 mg standard	NMT 1.00%	0.11%
Theoretical Plate Count for Pioglitazone	NLT 2000	3886

Linearity and Range

Under optimised condition the different concentration vs area was plotted in the range from 11.82 ppm to 35.46 ppm. The graph was found to be linear for concentration range and has been given in Figure 3. The data has been given in Tables 3 and 4.

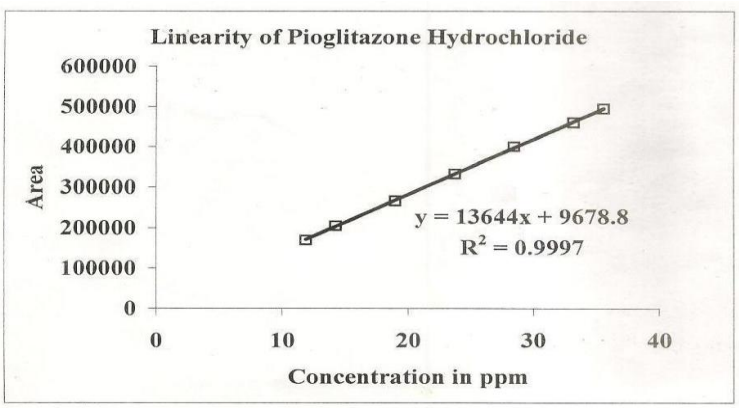

Figure 3: Calibration Curve (linearity of PiolitazoneHCl at 267nm)

Table 3: The results of Linearity

Sr. no	Volume taken (500 ppm)	Total Volume (ml)	Actual Conc (ppm)	Peak Area	Mean peak area

1	1	25	10	169275 170691 170804	170257
2	1.2	25	12	204612 206184 205966	205587
3	1.6	25	16	266659 265541 268638	266946
4	2	25	20	331110 334424 334826	333453
5	2.4	25	24	402532 400117 401734	401461
6	2.8	25	28	460898 460217 460124	460413
7	3	25	30	493432 494924 497108	495155
Slope				13644.42	
Intercept				9678.82	
Correlation coefficient (r^2)				0.99987	

Table 4: Values for Range`

Pioglitazone	**Level I Area**	**Level VII RT**	**Level I Area**	**Level VII RT**
	170643	7.99	500927	8
	170110	7.99	506426	8.01
	169748	8	501212	8
	170814	7.99	503589	7.99
	168114	7.99	506416	7.99
Mean	169886	7.99	503714	8
SD	1077.43	0.0045	2678.56	0.0084
%RSD	0.63	0.06	0.53	0.11

For precision studies; system precision as one of the parameter the sample was injected in 5 replicates and area, standard deviation and % RSD was calculated, it was expected and found to be less than 2% [32]. The data for system precision has been summarized in Table5, method precision in Table 6, interday and intra day precision in Table 7 and 8, and reproducibility in Table 10.

Table 5: The data for System precision

Std weight. (mg)	**Area**	**RT**	**Tailing**	**Theoretical Plates**
30.5	326346	8	1.85	3829

	324402	8	1.86	3862
	323473	8	1.85	3832
	320619	8	1.85	3874
	324888	8	1.83	3866
Mean	323946	8	1.85	3853
SD	2130.02	0	-	-
%RSD	0.66	0	-	-

Table 6: The results of method precision.

Sample wt. (mg)	Test Area	% Assay
200.2	352251	99.9
202.9	360322	100.9
200.3	352105	99.8
200.6	350623	99.3
202.1	357253	100.4
202.9	360433	100.9
	Mean	100.2
	SD	0.645
	%RSD	0.64

Table 7: The data for Interday Precision

Concentration (mg/ml)	Area		SD	%RSD
10	Day 1	171275	715.5028535	0.416175536
	Day 2	172691		
	Day 3	171804		
20	Day 1	332110	852.5311334	0.256436332
	Day 2	333424		
	Day 3	331826		
30	Day 1	494432	876.669455	0.177061539
	Day 2	494824		
	Day 3	496108		

Table 8: The data for system suitability

Concentration (mg/ml)	Area		SD	%RSD
10	1st Hour	169727	1677.66667	0.994010011
	4th Hour	169691		
	8th Hour	166804		
20	1st Hour	332100	2358.498675	0.710057526
	4th Hour	334542		
	8th Hour	329826		
30	1st Hour	482432	4271.592833	0.881599972
	4th Hour	489442		
	8th Hour	481708		

Table 9: Results for ANOVA test

Concentration (mg/ml)	Area for interday	Area for intraday
10	171275	169727
	172691	169691
	171804	166804
20	332110	332100
	333424	334542
	331826	329826
30	494432	482432
	494824	489442
	496108	481708
Results		
F	0.005172	
F crit	4.493998	

Table 10: The data for system suitability

Std wt. (mg)	Area	RT	Tailing	Theoretical Plates
30.2	311532	7.793	1.84	4520
	312515	7.72	1.88	4531
	311729	7.773	1.85	4582
	312154	7.729	1.83	4517
	312273	7.739	1.84	4593
Mean	312041	7.743	1.85	4549
SD	402.3969	0.0288	-	-
%RSD	0.13	0.37	-	-

Accuracy: For accuracy of the method, pioglitazone HCl was analyzed at three different levels in triplicates and the %RSD was calculated. The %RSD was found to be less than 2%.

Table 11: The results for accuracy

Level	Wt. of Placebo (mg)	Std wt. Level (mg)	Area	% Recovery	Mean Recovery (%)	SD	%RSD
80%	171.2	26.6	285631	100.3	100.1	0.2	0.2
	171.6	26.5	283474	99.9			
	171.5	26.4	283074	100.1			
100%	171.1	33.2	353232	99.3	99.5	0.3215	0.32
	171.8	33	352967	99.9			
	171.2	33.1	352258	99.4			
120%	171	39.8	425230	99.8	99.9	0.0577	0.06
	171.5	39.6	423806	99.9			
	171.1	39.9	426889	99.9			

Robustness

Under the selected experimental conditions, the standard and sample preparation run was carried out and % assay values were calculated. The data obtained for change in flow rate by +10% and -10% was summarised in Table 12 and Table 13 respectively. The data obtained for change organic phase by +10% and -10% has been summarised in table 14 and 15 respectively. And the data for change in pH +0.2 and -0.2 is summarized Table 16 and Table 17 respectively.

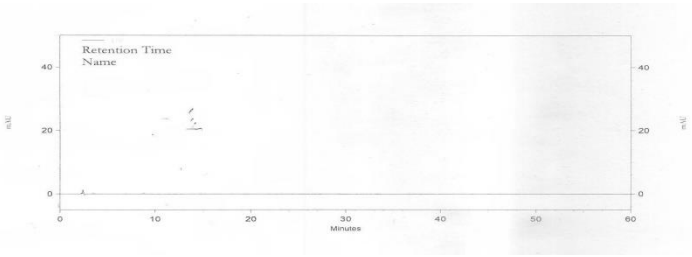

Figure 3: The chromatogram of the blank solution

Table 12: The results obtained for +10% flow rate in Robustness.

Sample weight in(mg)	Area	%Assay
200.2	314093	100.1
202.9	32004	100.7
200.3	313188	99.88
	Mean	100.2
	SD	0.4583
	%RSD	0.46

Table 13: The results obtained for -10% flow rate in Robustness.

Sample weight in(mg)	Area	%Assay
200.2	385904	100.4
202.9	390512	100.2
200.3	386249	100.4
	Mean	100.3
	SD	0.1155
	%RSD	0.12

Table 14: Results for %RSD for robustness (+10% organic phase)

Sample weight in(mg)	Area	%Assay
200.6	303435	100.6
202.7	302839	100.4
202.9	301986	100
	Mean	100.3
	SD	0.3055
	%RSD	0.3

Table 15: Results for % RSD for robustness (-10% organic phase)

Sample weight in(mg)	Area	%Assay
200.6	448627	99.2
201.4	452667	99.7
201.1	452914	99.9
	Mean	99.6
	SD	0.3606
	%RSD	0.36

Table 16: Results for RSD for robustness (pH+0.2 i.e. pH of buffer 5.2)

Sample weight in(mg)	Area	%Assay
202	355868	99.3
201.3	354766	99.3
202.9	359857	100
	Mean	99.5
	SD	0.4041
	%RSD	0.41

Table 17: Results for RSD for robustness (pH-0.2 i.e. pH of buffer 4.8)

Sample weight in(mg)	Area	%Assay
202.5	366165	100.3
202.1	362891	99.6
202.1	364282	100
	Mean	100
	SD	0.3512
	%RSD	0.35

CONCLUSION

The validated RP-HPLC assay method for pioglitazone can be used for determination of its purity.The method has been shown to be specific, linear, precise and accurate across a suitable analytical range for pioglatazone. Solutions have been shown to be stable for at least 24 hours on ambient storagecondition.This method was found to be better in consideration of other reported methods for individual drugs, because of economical readily available mobile phase, UV detection and better resolution of peak.This method will be advantageous for rapid quantification of sample in routine and quality control analysis for bulk and pharmaceutical dosage form containing pioglitazone.

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