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Research Article

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Analytical method development and validation of pioglitazone hydrochloride by RP-HPLC

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ABSTRACT

An accurate high-performance liquid chromatography with UV-visible detection (HPLC-UV) method for the determination of pioglitazone hydrochloride [(± pyridinyl) ethoxy] phenyl] methyl]-2,4-]thalizolidm.us.cominedionemono hydrochloride.it is an oral ant diabetic agent belonging to the class of thiazolidinediones. Isocratic separation of Pioglitazone is carried out using a reversed-phase Intersil ODS C18 (150 mm × 4.6 mm, 5µm) column with mobile phase consisting of Ammonium acetate buffer with Acetonitrile and Glacial acetic acid in the ratio 50:50:1 (v/v) and quantified by UV detection at 269 nm with flow rate of 0.7ml/min. Specific and reproducible HPLC method has been developed and validated for quantitative determination in Pioglitazone Hydrochloride This method is accurate, precise, linear and can be used for routine analysis of Pioglitazone Hydrochloride.

Keywords: Pioglitazone Hydrochloride.

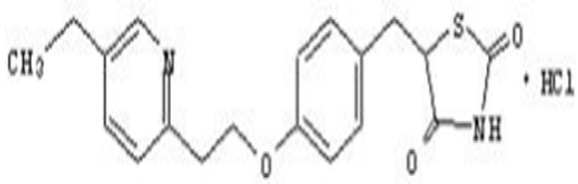
INTRODUCTION

Pioglitazone Hydrochloride [(± pyridine) ethoxy] phenyl] methyl]-2,4-] thalizolidinedione mono hydrochloride [24].it is an oral ant diabetic agent belonging to the class of thiazolidinediones that acts primarily by decreasing insulin resistance. It is a peroxisome proliferator-activated receptor γ(PPARγ) agonist that increases transcription of insulin-responsive genes and thus increases insulin sensitivity. It is used in the management of type 2 diabetes mellitus. It improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis also improves glycemic control while reducing circulating insulin levels. Pioglitazone [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-] thiazolidinedione monohydrochloride belongs to a different chemical class and has a different pharmacological action than the sulfonylureas, metformin, or the glucosidase inhibitors. It is used both as monotherapy and in combination with sulfonylurea or insulin in the management of type 2 diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM).

Pioglitazone is well absorbed after oral administration at doses ranging from 15 to 45 mg, peak concentrations of Pioglitazone in the blood of healthy subjects are achieved approximately 1.5 h after oral drug administration. Pioglitazone is highly bound to serum proteins (approximately 97%), with a low tissue distribution and slow elimination (half-life approximately 9 h). Pioglitazone is extensively metabolized in the liver, with the majority excreted as inactive metabolites in the feaces. Pioglitazone possesses a low molecular weight (MW; 392.90 Da) and a low aqueous solubility. The structure of Pioglitazone was shown in Fig. 1.

The aim of the present study is to develop a simple RP-HPLC method with UV detection for the quantitative determination of Pioglitazone hydrochloride. This method offers the advantage of simplicity with adequate sensitivity, selectivity, precision and accuracy. This analytical method can be used for the estimation of Pioglitazone hydrochloride in regular analysis.

Fig. 1: Structure of Pioglitazone hydrochloride



EXPERIMENTAL SECTION

Chemicals and reagents
Pioglitazone Hydrochloride pure form is obtained from SYN PURE laboratories, Hyderabad, India as gift samples. Methanol (HPLC grade), ammonium acetate, Acetonitrile, Glacial acetic acid, double distilled water were purchased from Merck. The working standard was obtained from SIGMA ALDRICH, Hyderabad, India.

Preparation of standard solution and test samples:
Standard Solution and sample solution was prepared in the concentration of 0.5mg/ml.

Instrumentation:
The HPLC system consisted of a Shimadzu LC- 2010 liquid chromatographic pump, Rheodyne injection port with a 20 µl sample loop UV-Visible spectrophotometer detector (Shimadzu, Kyoto, Japan). Data collection, integration and calibration were accomplished using Class VP chromatography Data system.

HPLC conditions;
The chromatographic determination of purity of Pioglitazone hydrochloride was accomplished using 150x4.6mm Intersil ODS C18 5µ reverse phase analytical column. The mobile phase consisted of methanol and ammonium acetate, acetonirile, Glacial acetic acid in the ratios of 50:50:1 v/v. Before use, the mobile phase was filtered by passing it through a 0.45µm filter and the filtrate is degassed by using ultrasonicator. The mobile phase was pumped at an isocratic flow of 0.7 ml/min at room temperature. The peaks were determined using a UV detector set at a wavelength of 269nm. All the procedures were performed at 25^{O}c column temperature.

EXPERIMENTAL PROCEDURE [1]_ [2]_ [3]**:**
Set the chromatographic conditions as stated above. First run the diluent for two injections to stable the baseline. Then inject the standard solution of 20µl injection volume and run it for six injections. Take the peak area values of six injections of working standard from the recorded chromatogram. Inject the sample solution of 20 µl injection volume and run it for three injections. Take the peak area values of three injections of sample injected from the recorded chromatogram.

ASSAY VALIDATION [19]-_ [23]**:**
The RP-HPLC assay validation was done as per ICH Q2A and Q2B guidelines. These tests included determination of Accuracy, Precision, Linearity, Specificity and Limit of detection, Limit of quantification, System suitability, Ruggedness, Robustness.

Specificity [4]_ [5]**:**
Inject 20µl of each solution individually the sample, standard, spike solutions and individually inject spike solution and develop chromatograms check the retention time of individual injections and spike samples in the chromatogram and observe whether the retention time is matching with that of standard. Details are given in table 1
Precision [12]-_ [18]**:**
Precision is carried out under system precision and method precision.

System precision Carried out the system precision and system suitability studies for Standard solution and test 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 are given in table 2.

Method precision [6]_ [7]**:**
Carried out the method precision studies for Standard solution with a minimum of six replicates of single preparation

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, details are given in table 3.

Accuracy (%recovery studies):
The accuracy studies are carried by preparing samples of 50%,100%,150%. The percentage recovery obtained is between 92.0% - 102%. Details are given in table 4.

Linearity [8]_ [9]**:**
The linearity studies are concentrations of 25% - 150% and the graph plotted is linear at this concentrations and co relation co efficient is found to be 0.999.details are given in table 5.

Range:
The range of concentration at 25% - 150% is found to be linear, accurate and precise.

Limit of detection & Limit of Quantification [10]_ [11]**:**
Standard solution is prepared of concentration of 1000ppm.from this stock solution of 500ppm is prepared and serial dilutions are prepared from 1 – 150% and LOD & LOQ is calculated by slope. Details are given in table 6:

Ruggeddness:
The studies are carried out by different analysts in different instruments by different analysts and %RSD is given in table 7:

Robustness:
The studies are by changing the parameters by changing column temperature, flow rate and at each case % RSD is given in table 8.

RESULTS AND DISCUSSION

Specific and reproducible HPLC method has been developed and validated for quantitative determination in Pioglitazone Hydrochloride.

ASSAY:
Standard graphs:

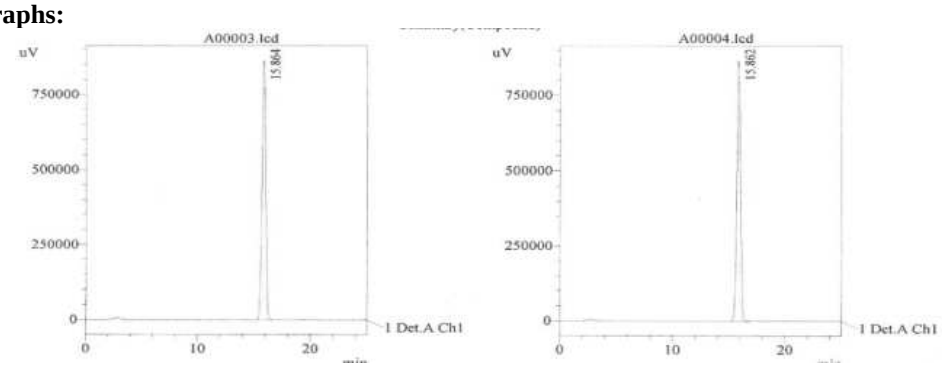
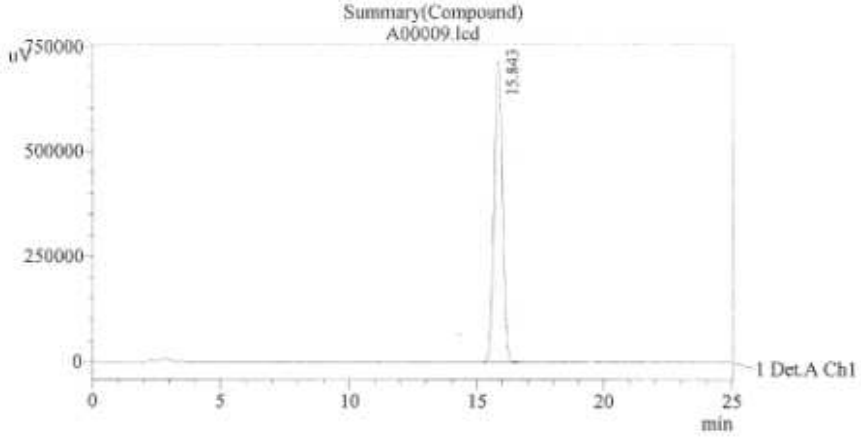


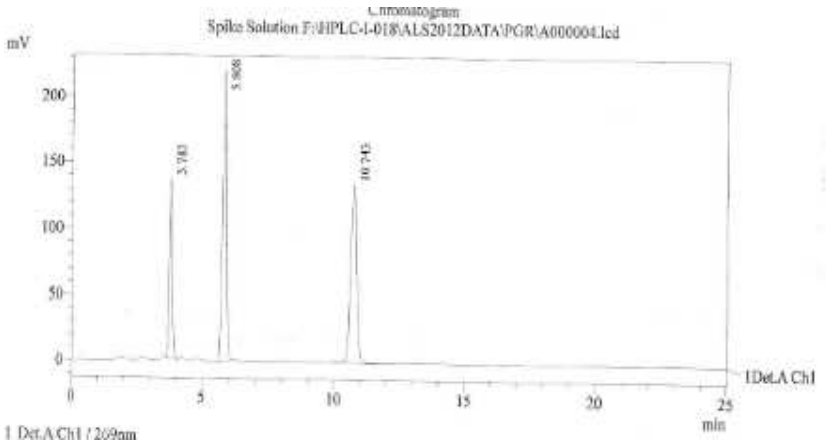
Table 1

S.No.	Sample name	RT	Area
1	Standard	10.766	11068663
2	Sample	11.224	5230749
3	M III	6.027	13761689
4	M IV	3.877	6728723

Sample graphs:



Percentge purity of Pioglitazone hydrochloride is found to be 99.47%.



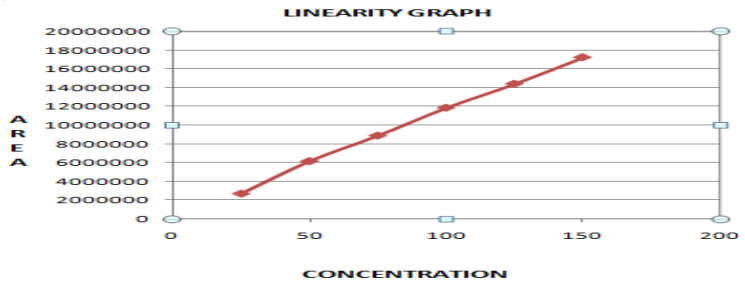


Table 2

S.NO.	Retention Time	Area	Tailing Factor	Theoretical Plates
1	10.797	11761386	1.024	9789.920
2	10.795	11774956	1.024	9778.583
3	10.796	11771082	1.025	9779.195
4	10.784	11769718	1.023	9806.395
5	10.780	11770818	1.023	9821.298
6	10.780	11773715	1.023	9801.849
Average	10.789	11770279.17	1.024	9796.207
Stdev	0.008	4775.268053	0.00	16.76
% RSD	0.02	0.04	0.00	0.06

Table 3

METHOD PRECISION				
S.No	**Batch no**	**Average area**	**Standard deviation**	**% RSD**
1	Standard solution	11207246.	74813.79	0.66
2	Test Solution	11969865	12717.45.	0.10

Table4

Accuracy			
S.No	**Concentration**	**Average Area**	**% of Recovery**
1	50%	4951452	93.34
2	100%	10244455.8	96.56
3	125%	15038620	94.50

Table 5

LOD& LOQ			
S.NO.	**Concentration (%)**	**Avg Area**	**Std dev**
1	1	48120	15.55634919
2	2	50574.5	123.7436867
3	5	148112	107.4802307
4	10	302725	14.14213562
5	25	622819.5	290.5081887
6	50	1177712.5	259.5081887
7	75	1785326	1217.637877
8	100	2490107.5	1058.538851
9	125	2899361.5	82.7314934
10	150	3578296	403.0508653
	Average Standard deviation		**357.3010565**
	Slope		**23580.826**
	LOD%	**0.05**	
	LOQ%	**0.15**	

Table 7

Summary of Ruggedness by % RSD				
Parameter	**Ref STD**	**Sample 1**	**Sample 2**	**Sample 3**
Analyst-1	0.03	0.09	0.02	0.05
Analyst-2	0.15	0.68	0.065	0.06

Table 8

Summary of Robustness by % RSD				
Parameter	**Ref STD**	**Sample 1**	**Sample 2**	**Sample 3**
30°C	0.34	0.03	0.13	0.04
40°C	0.18	0.17	0.21	0.35
0.5ml/min	0.04	0.05	0.41	0.02
1.0ml/min	0.73	0.02	0.05	0.08
Column-1	0.40	0.30	0.05	0.10
Column-2	0.04	0.03	0.002	0.30

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 C_{18} 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 10 min.

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