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Research Article

ANALYTICAL METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE ESTIMATION OF PIOGLITAZONE HYDROCHLORIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

Objective: To develop a new, accurate, precise and rapid isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method for the determination of Pioglitazone.

Method: Pioglitazone was analyzed on a BDS Thermohypersil C18 column (150x4.6mm, 5µ) was used with a mobile phase containing a mixture of Methanol and Ortho phosphoric acid buffer (pH-3) in the ratio of 40:60. The flow rate was 1ml/min and effluent was monitored at 266nm. The different HPLC experimental parameters were optimized and the method was validated according to standard guidelines.

Results: A peak area was obtained for Pioglitazone with 2.69min retention time. The calibration curve was linear over concentration ranges of 50-150 µg/ml of Pioglitazone. The lower limit of detection (LLOD) was found to be 0.806 µg/ml while their respective lower limit of quantification (LLOQ) value was 2.442 µg/ml. The average recovery was 99.33% for Pioglitazone. The percent of relative standard deviation (%RSD) of Pioglitazone was 0.1% for intra-day.

Conclusion: The method was found sensitive, accurate and precise. Hence the proposed method can be successfully applied in estimation of Pioglitazone in marketed formulation.

Keywords: Pioglitazone Hydrochloride, Reverse phase HPLC, Calibration curve, Pharmaceutical dosage form.

INTRODUCTION

Chemically, pioglitazone is (+)-5-(4-[2-(5-ethyl-2-pyridinyl) ethoxyl phenyl methyl]-2, 4- thiazolidinedione monohydrochloride. It improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis also improves glycemic control while reducing circulating insulin levels. It is used for the treatment of diabetes mellitus type 2 (previously known as non-insulin-dependent diabetes mellitus, NIDDM) in monotherapy and in combination with a sulfonylurea, metformin, or insulin.

Several HPLC methods have been reported for determining pioglitazone hydrochloride[1] in tablets. The quantitative determination of pioglitazone in human serum by direct-injection HPLC mass spectrometry and its application to a bioequivalence study has also been reported. Yamashita determined pioglitazone and its metabolites in human serum and urine and Zhang and Lakings reported an assay method for pioglitazone alone in dog plasma. Potentiometric sensors were fabricated for the determination of pioglitazone in some pharmaceutical formulations.

Drugs are available in tablet dosage from as pioglitazone 15mg (Pioglar) in the market. Literature survey revealed that pioglitazone has been estimated with other drugs[2,3] by UV[4] and HPLC[5,6].

MATERIALS AND METHODS

Reagents

Pioglitazone hydrochloride was kindly supplied by Dr. Reddy Labs (Hyderabad, A.P., India). Methanol (HPLC grade, Merck). Ortho phosphoric acid was purchased from Qualigens Fine Chemicals, Mumbai. Triethyl amine was purchased from S.D.Fine Chem. Ltd, Mumbai. All the other reagents were of AR grade.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer and methanol (60: 40, v/v). The buffer was prepared with 1000 ml water adjusted with ortho phosphoric acid to pH 2.5 + 0.1 and again adjusted with triethyl amine to pH 3 + 0.1. The buffer was filtered through a 0.45-µm (HVLP, Germany) membrane filter. The mobile phase was also filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Thermohypersil BDS C18 column (150 mm x 4.6mm, 5µm packing), was used for determination. The flow rate was 1.0 ml min-1 and the column was operated at ambient temperature (~25 °C). The volume of sample injected was 20 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 266 nm. A typical chromatogram of pioglitazone hydrochloride is shown in (Fig. 2).

Diluent: Methanol

Standard Preparation

Stock solution of Pioglitazone HCl was prepared by dissolving 50mg of Pioglitazone HCl in 50 ml volumetric flask add few ml of methanol to dissolve the drug and the volume is made up with methanol.

Sample Preparation

About 10 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and dose equivalent to 50mg was transferred to a 50 ml volumetric flask, dissolved in methanol and then the solution was made up to the mark with mobile Phase and filtered through 0.45 µ membrane filter. 5 ml of this solution was pipetted into 50ml volumetric flask and diluted with the methanol to get concentration of 100µg/ml.

Validation of method

The method developed here was validated as per ICH guidelines[7-9] for its accuracy, linearity, precision, specificity, robustness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table 10.

System suitability

System suitability and chromatographic parameters were validated such as number of theoretical plates, tailing factor and asymmetry factor were calculated.

Specificity and Selectivity

Specificity and selectivity were studied for the examination of the presence of interfering components. It was checked by subjecting the drug solution in different stress conditions like Acid, Base, Peroxide and the degradation was noted.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Pioglitazone HCl at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance and concentration of the drug. The response was found to be linear in the range 50-150 µg/ml for Pioglitazone HCl. The data was given in table 1.

Accuracy

Accuracy was performed in triplicate for various concentrations of Pioglitazone equivalent to 50%, 100% and 150% of the standard amount was injected into the HPLC system per the test procedure. The average % recovery of Pioglitazone HCl was calculated. The data was given in table 2.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure

B) Intermediate Precision (Analyst to Analyst variability)

Two analysts as per test method conducted the study. For Analyst-1 Method Repeatability and for Analyst-2 six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 3.

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Pioglitazone HCl was noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table 5. Ruggedness of the method was checked by using different analysts and instruments. The relative standard deviation of the results obtained from different analysts and instruments was <2.0%. The results were given in table 6 and 7.

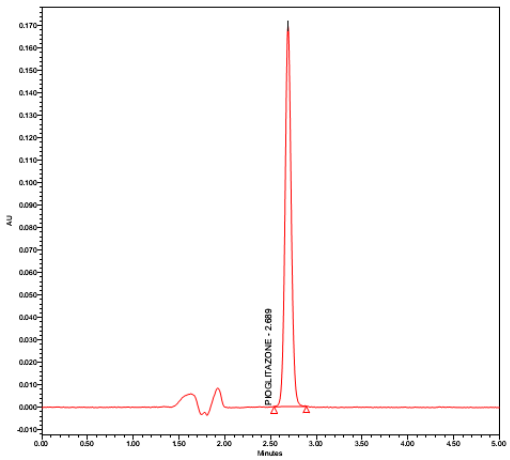

Fig. 1: It shows chromatogram of pioglitazone sample.

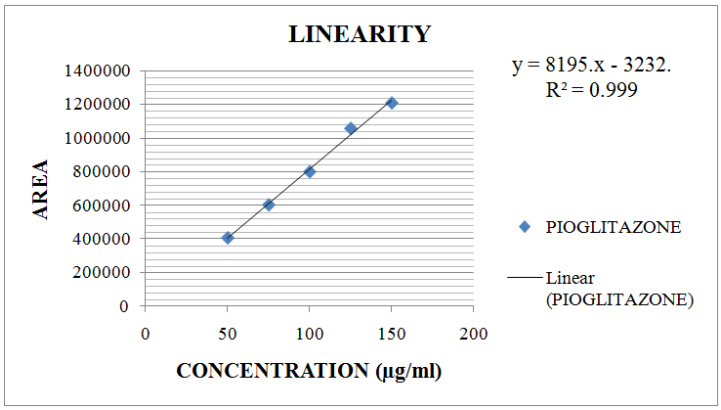

Fig. 2: It shows calibration curve of pioglitazone sample.

Table 1: It shows linearity of pioglitazone (n=3)

S. No.	Concentration (µg/ml)	Injection	Retention Time(mins)	Area
1	50	1	2.693	409808
2	75	2	2.693	605362
3	100	3	2.693	800964
4	125	4	2.691	1057291
5	150	5	2.693	1208321
y=8195x-3232 r^2=0.999				

Table 2: It shows accuracy (%recovery) of pioglitazone

S. No.	Spiked level	Amount Present (µg/ml)	Amount Added (µg/ml)	%Recovery	Std.Dev	%RSD
1(n=6)	50%	177.655	180.4227	98	5048	0.5
2(n=3)	100%	323.8333	320.26	101	12353	0.7
3(n=6)	150%	480.54	485.5933	99	9790	0.4

Table 3: It shows precision of pioglitazone (100µg/ml)

S. No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	100	1	2.689	802732
2	100	1	2.690	802683
3	100	1	2.690	801805
4	100	1	2.691	802883
5	100	1	2.691	802263
6	100	1	2.691	802420
Mean				802631
Std.Dev				551
%RSD				0.07

As a part of robustness, deliberate changes in the flow rate, mobile phase concentration, was made to evaluate the impact on the methods. Retention time was significantly changed with flow rate and mobile phase compositions (table 4 and 5).

Table 4: It shows robustness data relating to change in flow rate: (1.2ml/min)

S. No.	Flow rate (ml/min)	Injection	Retention time (min)	Area
1	flow rate-1-(1.1ml)	1	4.22	1832367
2	flow rate-2-(1..3ml)	1	4.02	1830235
Mean				1831301
Std dev				1508
%RSD				0.1

Table 5: It shows robustness data relating to change in mobile phase composition (Buffer: MeOH :: 50:50)

S. No.	Mobile phase	Injection	Retention time (min)	Area
1	m.p-1(49:51)	1	4.21	1835323
2	m.p-2(51:49)	1	2.58	1831152
mean				1833238
Std.Dev				2949
%RSD				0.2

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. LOD and LOQ were found to be 0.806µg/ml and 2.442 µg/ml respectively.

Table 6: It shows ruggedness data of pioglitazone Intraday

S. No.	Sample name	Injection	Retention time (min)	Area
1	Intraday-1	1	4.181	1834304
2	Intraday-2	1	4.161	1837299
3	Intraday-3	1	4.173	1835731
4	Intraday-4	1	4.159	1832070
5	Intraday-5	1	4.190	1833121
6	Intraday-6	1	4.165	1836883
Mean				1834901
Std.Dev				2093
% RSD				0.1

Instrument to Instrument

S. No.	Sample name	Injection	Retention time (min)	Area
1	INST-INST-1	1	4.172	1835420
2	INST-INST-2	1	4.184	1834210
3	INST-INST-3	1	4.191	1837263
4	INST-INST-4	1	4.165	1832120
5	INST-INST-5	1	4.179	1831314
6	INST-INST-6	1	4.184	1833004
Mean				1833889
Std.Dev				2208
% RSD				0.1

Acid Stress (0.1M HCl)

Table 7: It shows specificity testing (Acid stress) of pioglitazone

S. No	Pioglitazone					
	Concentration (µg/ml)	Time (hrs)	Retention time (min)	Area	% Assay	% Degradation
1	100	0	2.689	785206	100	
2	100	0	2.693	766325	96	-3

Base stress (0.1M NaOH)

Table 8: It shows specificity testing (Base stress) of pioglitazone

S. No.	Pioglitazone					
	Concentration (µg/ml)	Time (hrs)	Retention time (min)	Area	% Assay	% Degradation
1	100	0	2.689	784361	100	
2	100	24	2.692	730677	92	-7

Peroxide stress (0.1% H_2O_2)

Table 9: It shows specificity testing (Peroxide stress) of pioglitazone

S. No.	Pioglitazone					
	Concentration (µg/ml)	Time (hrs)	Retention time (min)	Area	% Assay	% Degradation
1	100	0	2.688	786533	100	
2	100	24	2.690	712246	89	-10

Table 10: It shows system suitability parameters of pioglitazone

Validation parameters	Pioglitazone HCl
Linearity range	50-150
Regression equation	Y = 8195x-3232
Correlation Coefficient(r^2)	0.999
Accuracy	99-100
Precision (%RSD)	0.07%
Ruggedness (%RSD)	
Intraday	0.2
Instrument to Instrument	0.4

RESULTS

A reverse-phase column procedure was proposed as a suitable method for the determination of pioglitazone dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of ortho phosphoric acid buffer (pH-3) and methanol in the ratio of 60:40 was used this mobile phase showed good resolution of Pioglitazone peak. The wavelength of detection selected was 266 nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Pioglitazone was about 2.69 minute and none of the impurities were interfering in its assay.

DISCUSSIONS

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate and can thereby easily adopted for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Pioglitazone in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. Many samples can be suitably analyzed for the routine analysis of Pioglitazone HCl in bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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