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Development and validation of HPLC and UV spectrophotometric methods for determination of pioglitazone hydrochloride in bulk and its formulations

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ABSTRACT

The present UV and HPLC methods are relatively simple, rapid and highly sensitive in the determination of pioglitazone hydrochloride (PIO). The aim of the present work was to develop and validate a simple, fast and reliable RP-HPLC and UV method for the determination of PIO in pharmaceutical dosage form. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature and dilution; short elution time; good precision (RSD less than 2) and high recovery (greater than 95%). Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonisation (ICH), to determination of PIO in pharmaceutical dosage form. Considering the possible world wide development of counterfeit pharmaceutical dosage forms, the proposed method could be useful for the quality control laboratories in developing countries.

Key Words: Pioglitazone Hydrochloride, HPLC, UV, Method Validation

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INTRODUCTION

PIO belongs to the class of thiazolidinediones. Chemically it is (±)-5-[p-[2-(5-ethyl-2-pyridyl) ethoxy] benzyl]-2, 4-thiazolidinedione monohydrochloride [1]

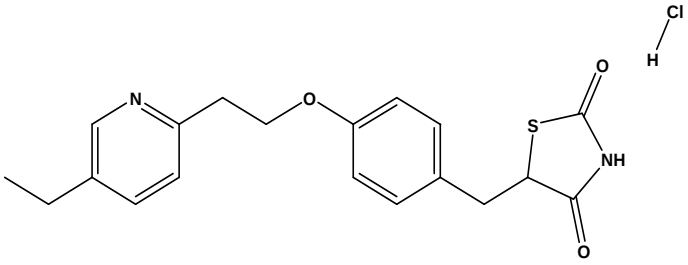

Fig.1: Chemical Structure of Pioglitazone Hydrochloride

PIO is as a potent and highly selective agonist for the peroxisome proliferator activated receptor-gamma (PPAR). It improves insulin response to target cells with increasing the pancreatic secretion of insulin. Literature survey revealed HPLC [4,5,7,9-11] HPTLC [8], Colorimetric [6] and UV methods in bulk drug and pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS/MS or GC-MS/MS that are complicated, costly and time consuming rather than a

simple HPLC_UV method. The present investigation by the author describes a simple, specific, rapid, accurate and precise RP-HPLC and UV methods for the determination of PIO from bulk samples and dosage form. The separation was effected on a C-18 column (250 mm X 4.6 mm; 5µ) using a mobile phase mixture of methanol: pH4.6 buffer in a ratio of 80:20 %v/v adjusted to pH 4.6 with 0.1 % v/v glacial acetic acid solution at a flow rate of 1.5 mL/min. The detection was made at 273 nm. The retention time of PIO was found to be 3.4 min. Calibration curve was linear over the concentration range of 5-30 µg/mL of PIO. In UV method, the wavelength selected for quantification was 269.2nm in 0.2M Hydrochloric acid. The linearity for detector response was observed in the concentration range of 5 to 40µg/mL. The results of the analysis have been validated statistically.

MATERIALS AND METHODS

Instrumentation and Analytical conditions

The HPLC method was performed by using Shimadzu Prominence binary gradient, high pressure liquid chromatographic instrument. The instrument was provided with a Phenomenex C18 (250 mm X 4.6 mm, 5µ), an LC 20 AD pump and an SPD 20A UV-Visible detector was employed in the study. A 20 µL Hamilton injection syringe was used for sample injection. Data acquisition was done by using LC solutions software. HPLC grade methanol and Analytical grade glacial acetic acid were used in the study. Triple distilled water used in the study was prepared in house using Borosil Glass Distillation Unit and Solar Distillation Unit. A freshly prepared binary mixture of methanol: pH4.6 buffer (80:20 %v/v) adjusted to pH 4.6 with 0.1 % v/v glacial acetic acid solution was used as the mobile phase. The mobile phase was filtered through a 0.45 µ membrane filter and degassed before use. The flow rate of mobile phase was maintained at 1.5 mL/min. The detection of the drug was carried out at 273 nm. The analysis was carried out at 22^{o}C.

The UV method was performed on a double-beam Shimadzu UV-Visible Spectrophotometer 1800, with spectral bandwidth of 1.0 nm, wavelength accuracy ± 0.1 nm and a pair of 1-cm matched quartz cells were used to measure absorbance of the resulting solution. Hydrochloric acid (Sd fine) and double distilled water were used as solvents in the UV method. Detection was done at 269.2 nm.

Drug samples

The reference sample of pioglitazone hydrochloride was supplied by M/s Aarti Drugs Ltd., Mumbai, India. The pharmaceutical dosage forms (tablets) - Pioglar-Ranbaxy (30 mg), PATH – Lupin (30 mg), were purchased from local market.

Preparation of stock and working standard solutions

HPLC Method

About 10mg of the PIO reference standard was weighed and transferred into 10 mL clean and dry volumetric flask. Then the volume was made up to the mark with the diluent and mixed well. This yielded standard stock solution with concentration 1000µg/mL of PIO. This solution was suitably diluted with the mobile phase to get a working standard solution of 100µg/mL of PIO.

UV Method

10 mg of PIO pure drug was accurately weighed and dissolved in minimum quantity of 0.2M HCl and diluted to 10 mL with same solvent. 1.0 mL of this solution was again diluted to 10 mL with the same solvent.

Linearity and Construction of Calibration Curve

HPLC Method

The quantitative determination of the drug was accomplished by an external standard method. The column was equilibrated with the mobile phase for at least 30 minutes prior to the injection of the drug solution. The Linearity (Beers's law) [2] of detector response is established by plotting a graph of concentration versus peak area of PIO standard and determining the correlation coefficient as shown in **Fig.2.**

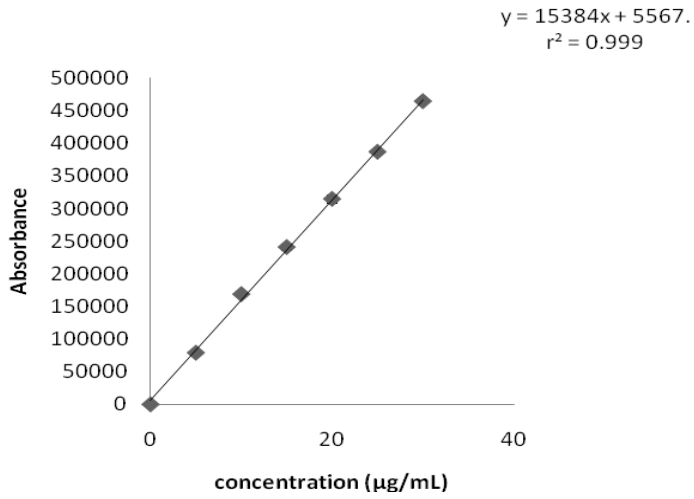

Fig. 2: Linearity of Pioglitazone Hydrochloride

A series of solution of PIO standard solution in the concentration ranging from about 5-30µg/mL level of the target concentrations were prepared and injected into the HPLC system as shown in **Fig.3**.

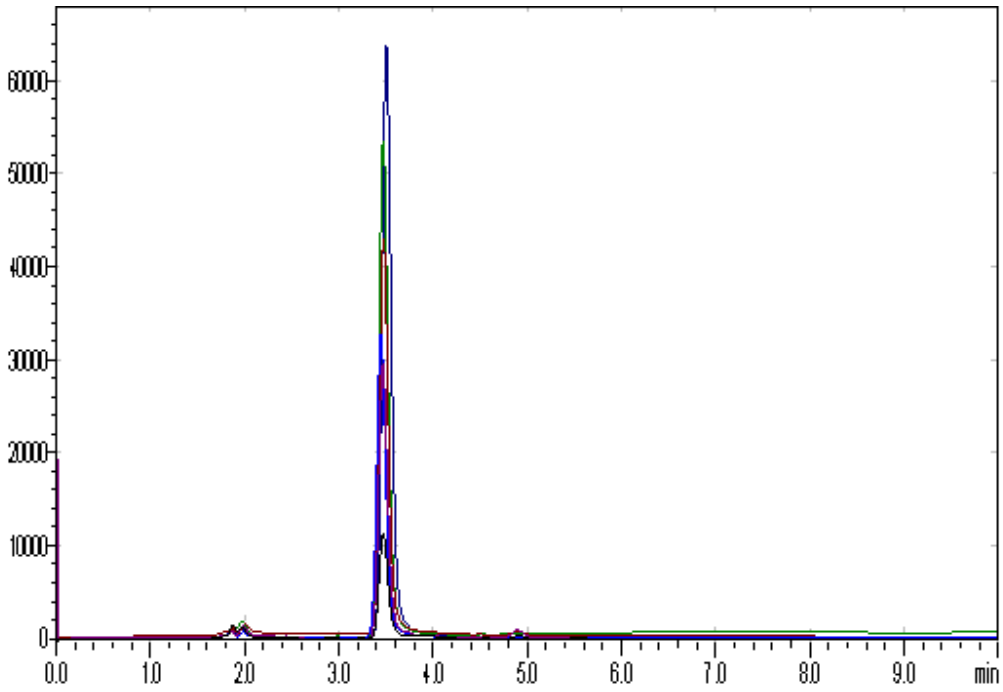

Fig. 3: Linearity of Pioglitazone Hydrochloride (5-30 µg/mL)

Each dilution was injected six times into the column. The drug in the eluates was monitored at 273 nm and the corresponding chromatograms were obtained. From these chromatograms, the mean peak areas were calculated and a plot of concentrations over the peak areas was constructed. The regression equation was later used to estimate the amount of PIO in pharmaceutical dosage forms. A representative chromatogram for the separation of PIO is given in **Fig.4**

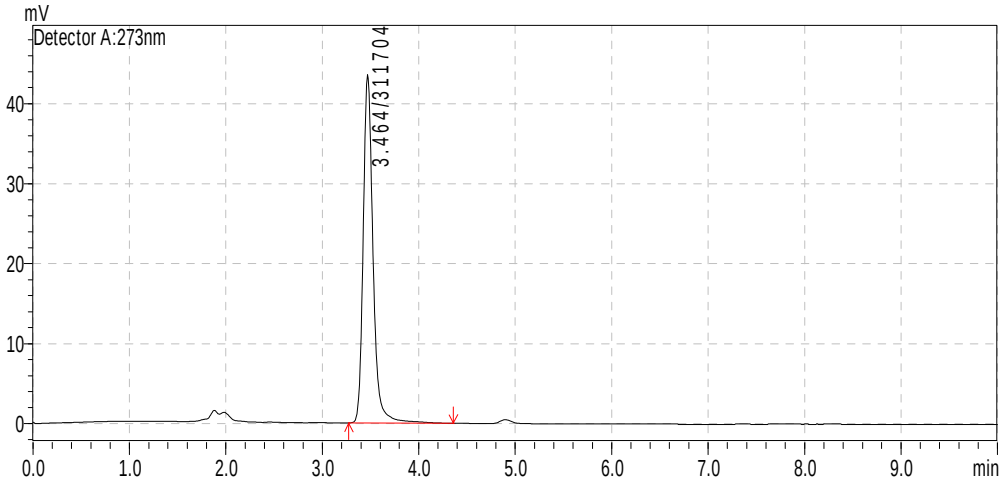

Fig. 4: A model chromatogram showing the separation of Pioglitazone hydrochloride

UV Method

Suitable aliquots of the standard solution of PIO (0.5 - 4.0 mL) were taken in 10 mL volumetric flasks. The volume was then made upto the mark with 0.2N hydrochloric acid to prepare a series of standard solutions containing 5 - 40 µg/mL. Absorbance was measured at 269.2 nm against blank **Fig.5**. Linearity graph is shown in **Fig.6**.

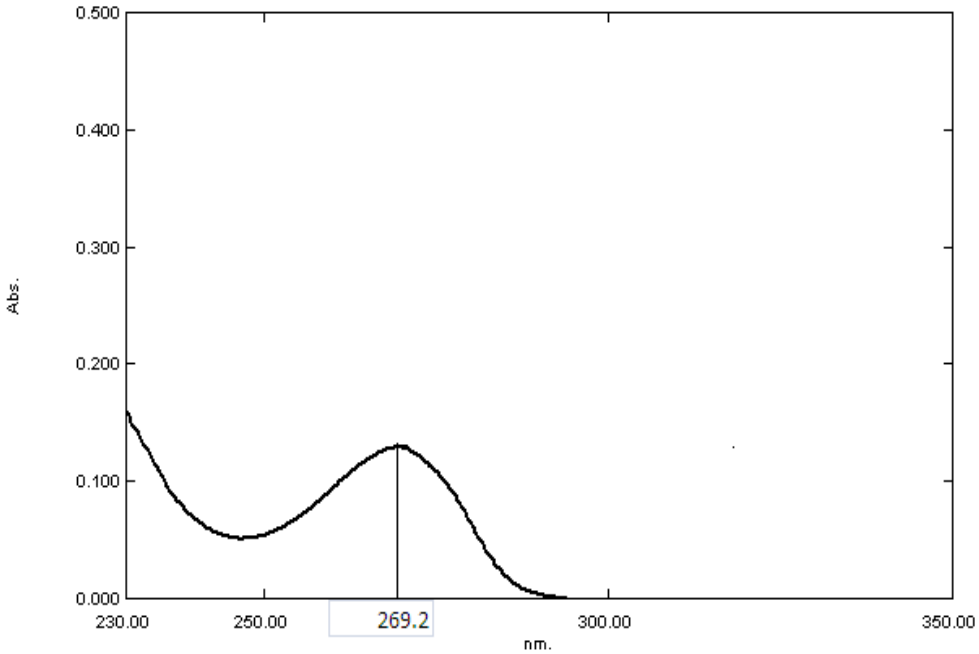

Fig. 5: Ultra Violet Absorption Spectrum of Pioglitazone Hydrochloride in 0.2N Hydrochloric acid at 269.2nm

Linearity was checked, the concentrations ranging from 5-40 µg/mL obeys Beer's law[2] **Fig.6**.

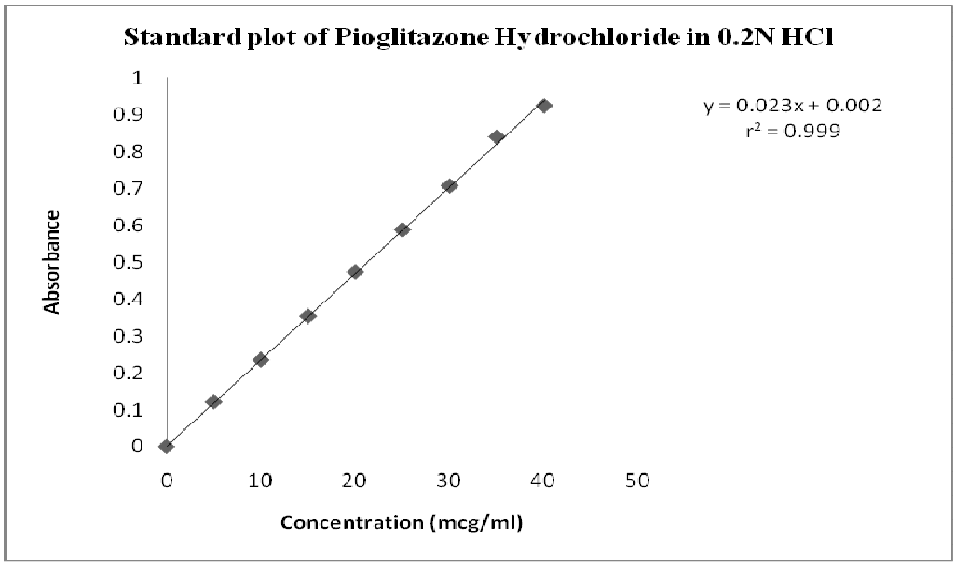

Fig.6: Calibration curve (Linearity) of Pioglitazone Hydrochloride at 269.2 nm

Preparation of sample solution

HPLC Method

Twenty tablets of two different formulations of PIO were taken and their average weight was calculated. The tablets were crushed to a fine powder, dose equivalent to 10 mg was transferred to a 10 mL volumetric flask, dissolved in methanol and then the solution was made upto the mark with mobile phase and filtered through 0.45µ membrane filter. 0.2 mL of this solution was pipetted into 20 mL volumetric flask and diluted with the mobile phase. The above solution was then injected six times into the column. The mean peak area of the drug was calculated from the chromatogram and the drug content in the formulation was calculated by the regression equation of the calibration plot. The results of the assay are shown in **Table.1**.

UV Method

Twenty tablets (of same respective batch number) of two pharmaceutical companies Pioglar (Ranbaxy), PATH (Lupin) were accurately weighed and powdered. Weight of powdered tablets equivalent to 10 mg of drug was taken in few mL of 0.2N HCl and vigorously shaken for 10 minutes, filtered through Whatmann filter paper No.41 and made up to 10 mL. 1 mL of the above solution was diluted to 10 mL with 0.2N HCl.

3.0 mL of above solution was transferred into a series of 10 mL volumetric flasks and the final volume was brought to 10 mL with 0.2N HCl. The absorbance was measured at 269.2 nm against 0.2N HCl as blank and the amount of PIO present in the sample solution was calculated. The experiment was repeated six times for each brand of tablets. Drug content in each brand of tablet was calculated and was recorded **Table.1**.

Table 1: Estimation of pioglitazone hydrochloride from its formulations

Method	Tablet Formulation	Label claim per tablet (mg)	% Label claim estimated (mean ± standard deviation)*	% RSD
HPLC	Pioglar	30	99.03±0.1803	0.181
	Path	30	100.72±0.1479	0.148
UV	Pioglar	30	99.96 ± 0.2902	0.2901
	Path	30	101.26 ± 1.5885	1.5687

** Average of six determinations; %RSD = percentage relative standard deviation*

METHOD VALIDATION [3]
HPLC& UV Methods

Accuracy
Accuracy (recovery) for the assay of PIO tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of PIO standard is added at different levels (80%, 100%, and 120%). The results of the recovery are shown in **Table. 2**.

Table 2: Results of Accuracy (Recovery) Studies

HPLC Method						UV Method			
Tablets Used	% Level of Recovery	Amount of the drug in tablet powder (mg)	PIO pure drug added (mg)	% Recovery Estimated	% RSD*	Amount of the drug in tablet powder (mg)	PIO Pure drug added (mg)	% recovery estimated	% RSD*
I (Pioglar)	80	15	12	100.37	0.64	15	12	100	0.57
	100	15	15	100.16	0.26	15	15	100.03	0.35
	120	15	18	100.06	0.39	15	18	100.33	0.36
II (PATH)	80	15	12	99.92	0.44	15	12	100.18	0.24
	100	15	15	100.1	0.43	15	15	100.13	0.33
	120	15	18	100.12	0.48	15	18	100.18	0.28

** Average of six determinations; %RSD = percentage relative standard deviation*

Precision
The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Intra day and inter day results are shown in **Table. 3**.

Table 3: Results of Precision

HPLC Method						
Concentration (µg/mL)	Intra-day Precision (n=6)			Inter-day Precision (n=6)		
	Amount found (µg/mL)	SD	%RSD*	Amount found (µg/mL)	SD	%RSD*
5	5.04	0.069	1.707	5.36	0.080	1.834
16	16.22	0.100	0.616	16.38	0.100	0.610
28	27.78	0.151	0.543	27.98	0.023	0.082
UV Method						
Concentration (µg/mL)	Intraday Precision (n=6)			Inter-day Precision (n=6)		
	Amount found (µg/mL)	SD	%RSD*	Amount found (µg/mL)	SD	%RSD*
10	10.4	0.116	1.115	10.83	0.028	0.258
20	21.05	0.086	0.408	20.40	0.023	0.112
30	31.07	0.075	0.241	30.64	0.057	0.186

n = number of measurements; SD = standard deviation; %RSD = percentage relative standard deviation
**Average of six determinations*

Linearity
From the standard stock solutions, a suitably mixed standard solution was prepared. The solutions were examined by the assay procedure. The calibration curve was plotted using concentration Vs absorbance of the standard solution. The slope and correlation coefficient values were calculated. The correlation coefficient of PIO was found to be 0.999 at 269.2nm. The calibration curve was plotted using concentration Vs absorbance of the standard solution. The calibration graph showed that a linear response was obtained over the range of concentrations used in the assay procedure. These data clearly demonstrates that the developed method have adequate sensitivity to the concentration of the analytes in the sample.

Limit of Detection (LOD) and Limit of Quantification (LOQ):
The LOD and LOQ were separately determined and reported, based on the calibration curve of standard solution. The relative standard deviation of the regression line or the standard deviation of y – intercepts of regression lines may be used to calculate LOD and LOQ. LOD = 3.3 × D/S and LOQ = 10 × D/S, where, D is the standard deviation of Y-intercepts of regression line and S is the slope of the calibration curve.

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of PIO was found to be 0.2638µg/mL. The LOQ is the lowest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of PIO was found to be 0.7995µg/mL.

RESULTS AND DISCUSSION

HPLC Method

The present study was aimed at developing a sensitive, simple, precise and accurate HPLC method for the analysis of PIO in bulk drug and pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixtures of methanol with buffer in different combinations were tested as mobile phase on C18 stationary phase. A binary mixture of methanol and pH4.6 buffer (80:20 %v/v) adjusted to pH 4.6 with 0.1 % v/v glacial acetic acid solution was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for PIO was 3.4 min. Each of the samples was injected six times and the same retention times were observed in all the cases. The peak areas of PIO were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed between the concentration of PIO and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was y = 15384 x + 5567 (where y gives peak area and x is the concentration of the drug). The absence of additional peaks indicated non-interference of common excipients used in the tablets is shown in **Fig.8** and **Fig.9.**

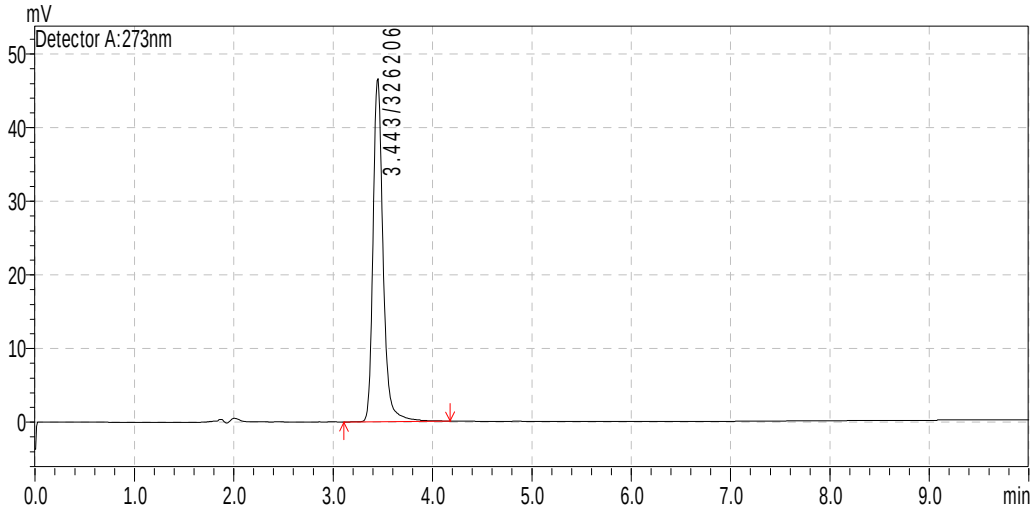

Fig. 8: HPLC Chromatogram of Pioglitazone hydrochloride Formulation I

High recovery values obtained from the formulations by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method. The tablets were found to contain and average of 101.32 % of the labeled amount of the drug. The low percentage relative standard deviation indicated the reproducibility of the assay of PIO in dosage forms. The results are given in table.

The deliberate changes in the method have not much affected the tailing factor, mean peak area and the retention time. This indicated the robustness of the method. The results are given in **Table.4**. System suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor, the number theoretical plates and resolution are all in the acceptable limits.

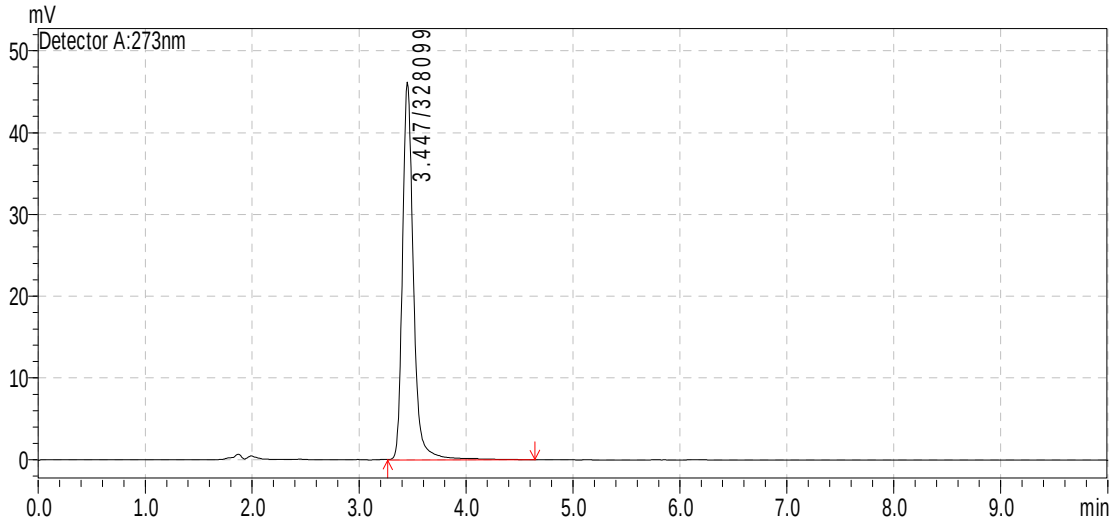

Fig. 9: HPLC Chromatogram of Pioglitazone hydrochloride Formulation II

Table 4: Robustness study

Factor	Level	Retention time(min)	Mean Area ± SD	Tailing factor
A. Flow Rate (mL/min)				
1.4	-10%	3.73	334250.3 ± 277.217	1.216
1.5	0	3.46	314605.7±5593.413	1.296
1.6	+10%	3.26	292678.7±1329.315	1.222
B. Percentage of Methanol in Mobile Phase				
79	-2%	3.6	313375.7±2869.278	1.217
80	0	3.46	314605.7±5593.413	1.296
81	+2%	3.26	310082±3261.431	1.231
C. pH of the Buffer				
4.4	-0.2	3.42	314966.7±3647.88	1.23
4.6	0	3.46	314605±5593.413	1.296
4.8	+0.2	3.21	315434±19572.43	1.247
D. Wavelength (nm)				
268	-5	3.36	346480±2409.407	1.245
273	0	3.46	314605±5593.413	1.296
278	+5	3.4	189274±13791.26	1.242

The system suitability results are shown in **Table.5**. The optimized chromatographic conditions are shown in **Table.6**.

Table. 5: HPLC System suitability parameters

S.No	Parameters	Value
1	Regression equation(Y)	15384x+5567
2	Correlation coefficient(r^2)	0.999
3	Slope(m)	15384
4	Intercept(C)	5567
5	Retention time(min)	3.4
6	Theoretical plates	>5000
7	Tailing factor	1.296
8	LOD(µg/mL)	0.0265
9	LOQ(µg/mL)	0.0805
10	Resolution	>2

Table. 6: Optimized Chromatographic Conditions

Parameter	Optimized condition
Chromatograph	Shimadzu HPLC with UV detector
Column	Phenomenex C-18 (250) column x 4.6mm, 5µ
Mobile Phase	Phosphate buffer: Methanol (20:80)
Flow rate	1.5mL/min
Detection	UV at 273nm
Injection volume	20µL
Temperature column	Ambient

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of PIO in pharmaceutical dosage form with short analysis time of less than 5 min.

UV Method

The solubility of PIO was determined in a variety of solvents. The proposed method is simple and precise and do not suffer from any interference due to common excipients of tablets. Method was validated in terms of accuracy, precision, LOD, LOQ and linearity. The accuracy of the method was proved by performing recovery studies in the commercially available formulations. Values greater than 99 % indicate that proposed method is accurate for the analysis of drug. The optical characteristic results are shown in **Table.7**.

Table 7: UV OPTICAL CHARACTERISTICS

Parameter	Value
λ_{max}	269.2nm
Beer's law limit (mcg/mL)	5-40
Regression equation (Y)	0.023x+0.002
Slope (m)	0.023
Intercept (c)	0.002
Correlation coefficient (r^2)	0.999
LOD (mcg/mL)	0.2638
LOQ (mcg/mL)	0.7995
Molar absorptivity(mol/lit/cm)	$0.9314X10^4$
Sandell's sensitivity(µg/ml 0.001 abs unit)	0.042644

The results for raw material and formulations in the proposed method were found to be satisfactory. In an over view the results indicate that the method is precise enough for the analysis of the drug.

CONCLUSION

A validated HPLC and UV methods have been developed for the determination of PIO in dosage forms. The proposed methods are simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 minutes allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of PIO in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS/MS or GC-MS/MS that are complicated, costly and time consuming rather than a simple HPLC_UV method. Considering the possible world wide development of counterfeit pharmaceutical dosage forms, the proposed method could be useful for the national quality control laboratories in developing countries.

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