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Original Article

SENSITIVE DETERMINATION OF RELATED SUBSTANCES IN PIOGLITAZONE HYDROCHLORIDE BY HPLC

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ABSTRACT

Objective: An efficient, high performance liquid chromatographic method has been developed and validated for the quantification of related substances in pioglitazone hydrochloride drug substance.

Methods: This method includes the determination of three related substances in pioglitazone hydrochloride. The mobile phase A is 0.1% w/v triethylamine in water with pH 2.5 adjusted by dilute phosphoric acid. The mobile phase B is premixed and degassed mixtures of acetonitrile and methanol. The flow rate was 1 ml/min. The elution used was gradient mode. The HPLC column used for the analysis was symmetry C18 with a length of 250 mm, the internal diameter of 4.6 mm and particle size of 5.0 microns.

Results: The developed method was found to be linear with the range of 0.006-250% with a coefficient of correlation 0.99. The precision study revealed that the percentage relative standard deviation was within the acceptable limit. The limit of detection and limit of quantitation of the impurities was less than 0.002%and 0.006% with respect to pioglitazone hydrochloride test concentration of 2000 µg/ml respectively. This method has been validated as per ICH guidelines Q2 (R1).

Conclusion: A reliable, economical HPLC method was magnificently established for quantitative analysis of related substances of pioglitazone hydrochloride drug substance.

Keywords: Pioglitazone hydrochloride, Related substances, HPLC, Validation

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INTRODUCTION

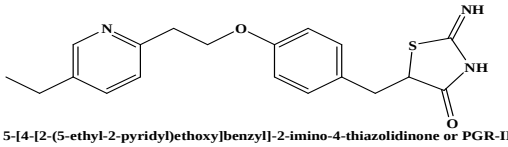
Pioglitazone is a prescription drug of the thiazolidinedione class with hypoglycemic action to treat diabetes. Pioglitazone is used to lower blood glucose levels in the treatment of diabetes mellitus type 2 either alone or in combination with a sulfonylurea, metformin, or insulin. Pioglitazone has also been used to treat non-alcoholic steatohepatitis [1-2].

The related substances of pioglitazone hydrochloride have been developed and validated as per the selection of the synthetic route. Nowadays, the regulators were very much interested about the strategy of the control of impurities present in drug substances. They insist to have a regulation on impurities in each step of the synthetic drug process. Many studies were explained about the determination of pioglitazone was performed by HPLC [3-25, 27-31]. So as to determine the related substances of pioglitazone hydrochloride, the research work has been initiated.

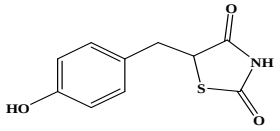
Several methods have been developed and validated only for the content of pioglitazone hydrochloride in drug substances or drug products and not for their related substances [3-25, 28-31]. This has been triggered us to perform the development activity for the determination of related substances in pioglitazone hydrochloride by HPLC and their structures were shown in fig. 1. As per the literature survey of the pioglitazone hydrochloride, no one has reported the most sensitive method for the determination of three impurities in pioglitazone hydrochloride drug substance by HPLC. These three impurities were determined at very low-level detection and quantitation in pioglitazone hydrochloride drug substance and this comprises the novelty of the article.

MATERIALS AND METHODS

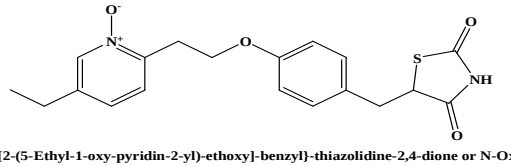
Pioglitazone hydrochloride, PGR-II, PIO-II, N-oxide were gifted by Techno chemicals. The structure of related substances and pioglitazone hydrochloride has shown in fig. 1. Triethylamine, methanol, phosphoric acid and acetonitrile were bought from Fisher scientific. HPLC grade water was used, equipped with the Elga water purification system, Metrohm. Transferred 1 ml of triethylamine in 1000 ml water, adjusted the pH of the solution to 2.5 using dilute orthophosphoric acid, filtered and degassed [27-31]. This solution was named as mobile phase-A. Mixed 800 ml of acetonitrile and 200 ml of methanol, degassed and used as mobile phase-B. Water bath equipped with a controller (Amkette analytics, ANM alliance) was used for forced degradation studies. Photolytic studies were carried out in a photostability chamber (Thermolab photostability chamber, India). Thermal degradation works were accomplished in a hot air oven (Amkette analytics, ANM alliance).



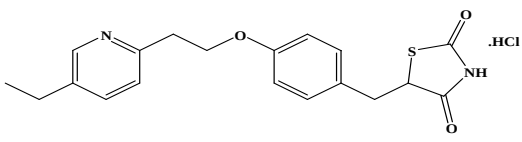
5-[4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl]-2-imino-4-thiazolidinone or PGR-II



5-(4-Hydroxy-benzyl)-thiazolidine-2,4-dione or PIO-II



5-{4-[2-(5-Ethyl-1-oxy-pyridin-2-yl)-ethoxy]-benzyl}-thiazolidine-2,4-dione or N-Oxide



5-{4-[2-(5-Ethyl-pyridin-2-yl)-ethoxy]-benzyl}-thiazolidine-2,4-dione, hydrochloride or Pioglitazone hydrochloride

Fig. 1: The structures of related substances and pioglitazone hydrochloride

Preparation of solutions

The sample diluent was prepared by diluting 8.5 ml of concentrated hydrochloric acid in 1000 ml of water. Filtered and degassed for usage of analysis. The system suitability solution was prepared by exactly weighed and transferred about 10 mg each of PIO-II, PGR-II, N-oxide and pioglitazone standard in 100 ml volumetric flask. Dissolved and make upto the volume 100 ml with sample diluent. Further, 2 ml of this solution was diluted into a 100 ml volumetric flask and made up to the mark with sample diluent. So, the standard solution concentration was 100 µg/ml with respect to the test concentration of 2000 µg/ml pioglitazone hydrochloride. The sample solution was prepared by accurately weighed and transferred about 100 mg of sample into 50 ml volumetric flask. Dissolved and make upto the volume 50 ml with sample diluent. The chromatograph of system suitability solution has been shown in fig. 2.

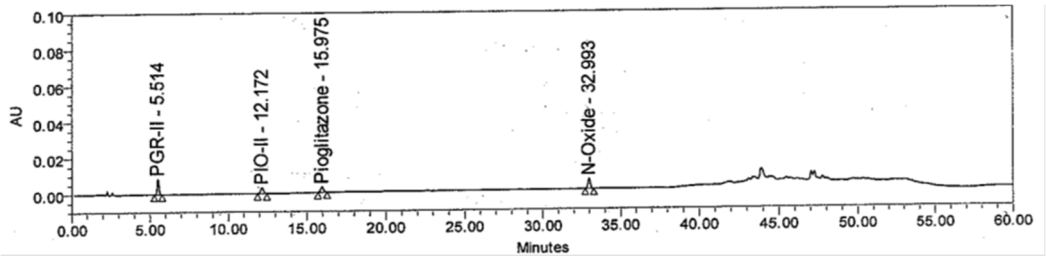


Fig. 2: Chromatograms of system suitability solution

Concluded method for validation purpose

The HPLC column was used symmetry C18, 250 mm x 4.6 mm x 5.0 µm. Transferred 1 ml of triethylamine in 1000 ml water, adjusted the pH of the solution to 2.5 using dilute orthophosphoric acid, filtered and degassed. This solution was named as mobile phase-A. Mixed 800 ml of acetonitrile and 200 ml of methanol, degassed and used as mobile phase-B. The gradient program was mentioned as min/%B composition; 0.00/20.0, 10.00/20.0, 35.00/40.0, 40.00/ 80.0, 50/80.0, 51.00/20.0 and 60.00/20.0. The flow rate was 1 ml/min. The wavelength of detection was 225 nm and the injection volume was 10 µl. The column compartment temperature was maintained at 45 °C.

RESULTS

Analytical method development

Several methods have been developed by HPLC for the determination of pioglitazone in the bulk and formulated products [3-25, 28-31]. These previously published research articles were failed to explain about the determination of related substances in pioglitazone. This mainly leads us to do further development of the method with three impurities in pioglitazone by HPLC.

The same column [3] has been used in the initial development. So, as to develop the sensitive method, the mobile phase, pH and gradient composition have been modified accordingly. The pH of the mobile phase was maintained in the acidic region, which was achieved by the addition of 1 ml of triethylamine in 1000 ml of water, adjusted the solution pH to 2.5 using dilute phosphoric acid. Generally, the mass compatible mobile phase needs to be selected, because if any impurities were detected at the level of above or below LOQ in the sample which needs to be confirmed using HPLC-MS study. But the mobile phase chosen for the study was not compatible with mass spectrometry (MS). So, this could be the limitation of the present research work. These three impurities have been eluted and separated well within the time of 40 min., but to ensure the consistency and specificity of the other impurities which was mentioned in the research article [27-31]; the gradient program has been slightly modified and extended up to 60 min. From this trial, the method was specific for all the impurities which have mentioned in the research article [27-31]. The results and comparisons of previously published articles were given in table 1.

Table 1: Reported analytical HPLC methods for determination of pioglitazone (PIO) either alone or in combination with other drugs like metformin (MET), glimepiride (GLM), rosiglitazone (ROS) and gliclazide (GLC) in pharmaceutical dosage forms.

Reference	Study Aim	Mobile phase	Column	Wavelength (nm)	Flow rate (ml/min)	LOD (µg/ml)
3	In bulk and pharmaceutical formulations by HPLC and MEKC method	0.01M KH_2PO_4 buffer (pH 6.0):ACN (50:50, v/v)	Symmetry C18 (250 mm x 4.6 mmx 5 µm)	225	1	-
4	SIAM by RP-HPLC	Phosphate buffer (pH 4.0), ACN and methanol (55:30:15, v/v)	Prontosil C8 (250 mm x 4.6 mm x 5 µm)	254	1.5	-
5	Study of stressed degradation behaviour in bulk and pharmaceutical formulation	0.01M KH_2PO_4buffer (pH 3.5)and methanol (55:45, v/v)	Phenomenex Luna C18 (250 mm x 4.6 mm x 5 µm)	241	1.5	1.69
6	Assay of tablets	Ammonium formate buffer (pH 3): ACN (75:25, v/v)	Nova-Pak C18 (150 mm x 3.9 mm x 5 µm)	225	1	-
7	Purity test and assay of tablets	Ammonium formate buffer (pH 4.1): ACN (45:55, v/v)	Symmetry C18 (250 mm x 4.6 mm x 5 µm)	266	1	0.042
8	SIAM	ACN: (0.15, v/v) triethylamine (pH 4.6) (40:60, v/v)	Hypersil C-8 (250 mm x 4.6 mm x 5 µm)	220	1.5	0.6
9	Simultaneous determination with GLM	0.01M triammonium citrate (pH 6.95):ACN: MeOH (45:35:20, v/v/v)	Cosmosil C18 (150 mm x 4.6 mm x 5 µm)	228	1	-
10	Simultaneous with MET	ACN: KH_2PO_4 buffer (pH 3) (50:50, v/v)	Hypersil BDS C18 (250 mm x 4.6 mm x 5 µm)	238	1	-

11	Simultaneous determination with GLM	ACN: 0.02 M Ammonium acetate buffer (pH 4.5) (60:40, v/v)	Inertsil ODS (250 mm x 4.6 mm x 5 µm)	230	1	0.2
12	Simultaneous determination with MET and GLM in tablet formulation	MeOH: KH_2PO_4 buffer (pH 4.3) (75:25, v/v)	Inertsil ODS-3 C18 (250 mm x 4.6 mm x 5 µm)	258	1	-
13	Simultaneous determination with saxagliptin in tablets	ACN: 0.02 M KH_2PO_4 buffer (pH 7.0) (60:40, v/v)	Inertsil C18 (150 mm x 4.6 mm x 5 µm)	260	0.8	0.010
14	Simultaneous determination with GLM	ACN: 0.01 M KH_2PO_4 buffer (pH 6.2) (50:50, v/v)	Eurosphere-100 C18 (250 mm x 4.6 mm x 5 µm)	225	1.4	0.00049
15	Simultaneous determination with GLM and ROS	Dil. H_3PO_4 (pH 3.0): ACN (80:20, v/v)	Nucleodur C-18 (250 mm x 4.6 mm x 5 µm)	215	0.8	0.19
16	Estimation along with MET in tablets	ACN: water: acetic acid (75:25:0.3, v/v/v), pH 5.5	Hypersil ODS C18 (250 mm x 4.6 mm x 5 µm)	230	0.5	0.009
17	Simultaneous quantification with GLM and MET	ACN: 0.01 M KH_2PO_4 buffer-pH 5.0:THF (50:40:10, v/v/v)	Inertsil ODS 3V (250 mm x 4.6 mm x 5 µm)	228	1.7	-
18	Simultaneous estimation along with GLM	ACN: KH_2PO_4 buffer (60:40, v/v)	Inertsil ODS (150 mm x 4.6 mm x 5 µm))	225	1.5	0.12
19	SIAM along with GLM	Solution A: ACN Solution B: 0.02 M KH_2PO_4 buffer (pH: 3.2)	Zorbax cyano (250 mm x 4.6 mm x 5 µm)	230	0.8	-
20	Simultaneous determination with MET and GLC in multicomponent formulation	MeOH: 0.02 M KH_2PO_4 buffer (85:15, v/v)	HiQSilC18 HS (250 mm x 4.6 mm x 5 µm)	227	1.2	0.1
21	Simultaneous estimation with GLM	Methanol: water (72:28, v/v)	AgilentTC–C18 (250 mm_4.6 mm,5 µm)	230	1	0.760
22	Simultaneous estimation with telmisartan	ACN: ammonium dihydrogen phosphate (pH 4.5; 0.02 M) (65:35, v/v)	Phenomenex C8 (250 mm_4.6 mm,5 µm)	210	1	0.82
23	Determination of along with MET and GLM	ACN: phosphate buffer (pH 3) (65:35, v/v)	PhenomenexRP-18 (150 mm_4.6 mm,5 µm)	245	0.5	0.061
24	Micellar liquid chromatographic analytical method for determination of atorvastatin calcium	Tween-20: n-butanol: phosphate buffer, (pH 4.2) (50:25:25,v/v/v)	Luna C18 (250 mm_4.6 mm,5 µm)	322	1.5	-
25	HPLC	0.01 M buffer, pH-6.0:methanol (40:60, v/v)	Symmetry–extend–C18 (150 mm_4.6 mm,5 µm)	240	1.2	-
27	SIAM for determination of impurities in PIO	Sol-A: phosphate buffer pH 3.1 and Sol-B: acetonitrile	Inertsil ODS-3V (150 mm_4.6 mm,5 µm)	225	1.5	Impurity-B: 0.033
Present work	HPLC	Sol. A: 0.1% w/v triethylamine, pH 2.5 Sol. B: ACN: MeOH (80:20) (v/v)	Symmetry–C18 (250 mm_4.6 mm,5 µm)	225	1.0	LOQ: PGR-II: 0.00041 PIO-II: 0.000118 N-oxide: 0.000064 Pioglitazone: 0.000109

ACN: Acetonitrile; MeOH: Methanol; LOQ: Limit of quantitation; LOD: Limit of detection

Analytical method validation

System suitability, system precision, method precision, detection limit, quantitation limit, linearity with regression and range, recovery, specificity/stress study, robustness and solution stability have been accomplished in the method validation study [26].

System suitability

The system suitability solution was injected and calculated USP resolution for each peak. USP resolution was obtained above 5.0; the results were shown in table 2. (Limit: USP resolution should be more than 5.0 for each peak). The system precision results have been given in table 2.

Table 2: System suitability results of PGR-II, PIO-II, N-oxide and pioglitazone

Peak name	RT (min)	RT ratio	USP resolution*
PGR-II	5.51	0.35	-
PIO-II	12.17	0.76	23.58
Pioglitazone	15.98	1.00	10.91
N-oxide	32.99	2.07	53.53

RT: Retention time; USP: United states pharmacopoeia; *USP resolution between any peaks should be more than 1.5.

System precision

The precision of an analytical procedure: expresses the nearness of treaty amongst a sequence of quantities obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [26]. The standard solution was repeatedly injected and performed the calculation of % RSD for each peak. %RSD was obtained was less than 0.6% (limit: %RSD should be less than 4%). The system precision results have been given in table 3.

Table 3: System precision results of PGR-II, PIO-II, N-oxide and pioglitazone

Injection no.	Area observed			
	PGR-II	PIO-II	N-oxide	Pioglitazone
1	70906	40674	58335	38210
2	71177	40876	58328	38367
3	71172	40853	58556	38487
4	70910	40883	58194	38191
5	70547	41004	58160	38428
6	70587	40946	58018	38168
7	70481	40801	58157	38333
8	70493	41295	58075	38748
9	70536	40588	57914	38697
10	70433	41054	58010	38413
Mean	70724	40897	58175	38289
SD	289.8593	198.2984	190.0509	199.1207
%RSD*	0.41	0.48	0.33	0.52

SD: Standard deviation; RSD: Relative standard deviation; * %RSD limit for 10 injections were should be less than 4%

Method precision

The precision of an analytical procedure: expresses the nearness of treaty amongst a series of quantities attained from several sampling of the identical homogeneous samples under the prescribed conditions [26]. The method precisions have been performed by six preparations of spiked sample solutions with impurities of PGR-II, PIO-II and N-oxide. The %RSD for the content of PGR-II, PIO-II and N-oxide was below 6% (limit: %RSD for content should be less than 10%). The %RSD for the content of impurities was within 6% in the intermediate precision which was performed by different analysts, column, instrument and day. The table 4 shows the results of method precision data.

Limit of detection and limit of quantitation

The limit of detection and limit of quantitation was examined based on signal-to-noise ratio method as per the ICH guideline Q2 (R1). The signal to noise ratio for a limit of detection is 3:1 and the limit of quantitation is 10:1. This was performed by performing the sequence of dilute solutions with a known concentration limit of detection and limit of quantification has been determined.

The limit of detection for PGR-II, PIO-II, N-oxide and pioglitazone were 0.0007, 0.0020, 0.0018, and 0.0011% respectively. The limit of quantification for PGR-II, PIO-II, N-oxide and pioglitazone were 0.0020, 0.0059, 0.0054, 0.0032 % respectively.

Table 4: Method precision results of PGR-II, PIO-II and N-oxide

Preparation no.	% of PGR-II	% of PIO-II	% of N-oxide
1	0.10	0.10	0.10
2	0.10	0.09	0.10
3	0.10	0.09	0.10
4	0.10	0.10	0.10
5	0.10	0.10	0.10
6	0.10	0.10	0.10
Mean	0.10	0.10	0.10
%RSD*	0.0	5.2	0.0

RSD: Relative standard deviation; *% RSD for content of impurities was should be less than 10%

Table 5: Linearity data of PGR-II

Sample No.	% Level	Concentration (µg/ml)	Peak response
1	LOQ	0.00041	1653
2	30	0.000616	22562
3	50	0.001026	37490
4	100	0.002052	75191
5	120	0.002462	80592
6	200	0.004104	151625
7	250	0.005130	185604
Slope		36295198.1479	
Y-intercept		-764.8861	
Multiple R		0.9985	
R square		0.9971	

LOQ: limit of quantitation

Linearity

The linearity of the analytical procedure: is its capability to attain assessment outcomes which are straightly proportional to the concentration of an analyte in the sample [26]. Linearity was performed from LOQ to 250% of 2000 µg/ml analyte concentration. The correlation coefficient values have been shown in the table 5-8. The linearity graphs were shown in fig. 3-6. The values of multiple R and R-square were almost equal to one; this indicates that the developed method was linear. The regression results indicate that the validated method was linear over the total concentration and it was satisfactory for its concentration range from LOQ to 250%. The R-square and multiple R values indicate that the method was linear and it was very close to the origin or close to the ideal theoretical value.

Table 6: Linearity data of PIO-II

Sample No.	% level	Concentration (µg/ml)	Peak response
1	LOQ	0.000118	2563
2	30	0.000600	13031
3	50	0.001000	21339
4	100	0.002000	43834
5	120	0.002400	51729
6	200	0.004000	86483
7	250	0.005000	105319
Slope		21218800.9574	
Y-intercept		501.7382	
Multiple R		0.9998	
R square		0.9995	

LOQ: limit of quantitation

Table 7: Linearity data of N-oxide

Sample No.	% level	Concentration (µg/ml)	Peak response
1	LOQ	0.000064	2388
2	30	0.000597	20226
3	50	0.000996	33079
4	100	0.001992	68478
5	120	0.002390	80713
6	200	0.003983	134433
7	250	0.004979	165330
Slope		33349222.1603	
Y-intercept		625.0455	
Multiple R		0.9999	
R square		0.9997	

LOQ: limit of quantitation

Table 8: Linearity data of pioglitazone

Sample No.	% Level	Concentration (µg/ml)	Peak response
1	LOQ	0.000109	2362
2	30	0.000605	14950
3	50	0.001008	23884
4	100	0.002016	49743
5	120	0.002420	57282
6	200	0.004033	96295
7	250	0.005041	119350
Slope		23707392.2216	
Y-intercept		393.5717	
Multiple R		0.9998	
R square		0.9997	

LOQ: limit of quantitation

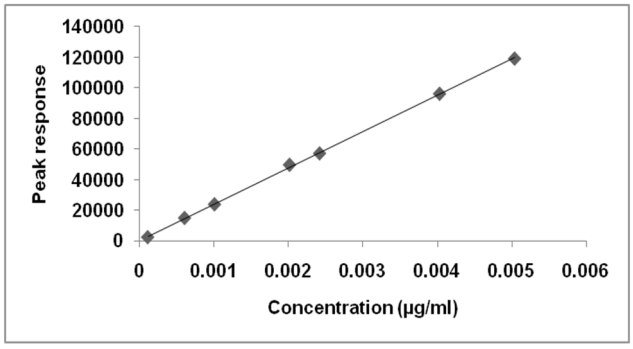


Fig. 3: Linearity graph for PGR-II

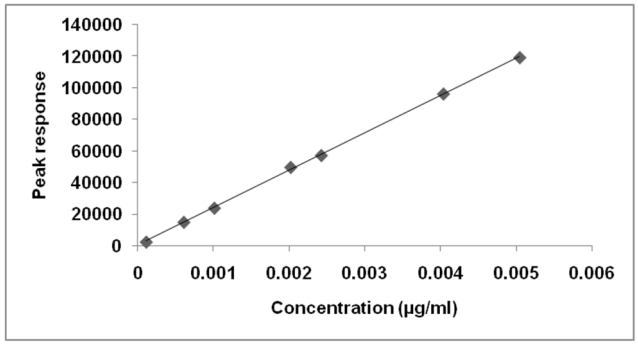


Fig. 4: Linearity graph for PIO-II

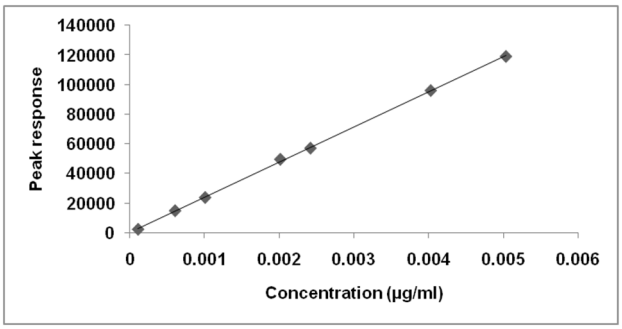


Fig. 5: Linearity graph for N-oxide

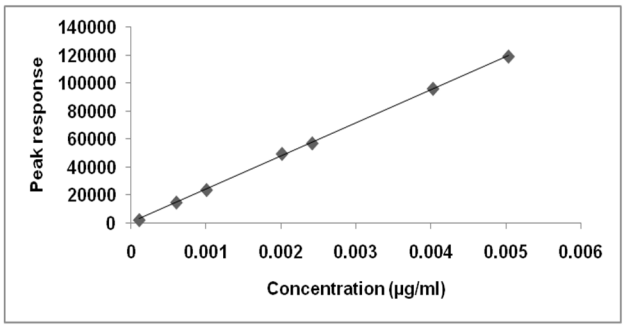


Fig. 6: Linearity graph for pioglitazone

Regression

The regression parameters of PGR-II, PIO-II, N-oxide and pioglitazone have been summarized in table 9. This explains about the statistical evaluation of ANOVA, confidence intervals and intercepts value with respect to 100% standard concentration response.

Range

PGR-II, PIO-II, N-oxide and pioglitazone were established in the range of 2.0-256.5%, 5.9-250.0%, 3.2-250.2% and 5.5-252.1% respectively.

Table 9: Regression statistics

PGR-II	**ANOVA**	
	Degree of freedom (df)	**Sum of squares (SS)**
Regression	1	27465435079.1892
Residual	5	80876504.2393
Total	6	27546311583.4286
PGR-II	**Confidence Intervals**	
	Lower 95%	**Upper 95%**
Intercept	-7103.8007	5574.0285
X Variable 1	34031004.6031	38559391.6926
PIO-II	**ANOVA**	
	Degree of freedom (df)	**Sum of squares (SS)**
Regression	1	8772141115.7928
Residual	5	4127381.6358
Total	6	8776268497.4286
PIO-II	**Confidence Intervals**	
	Lower 95%	**Upper 95%**
Intercept	-942.2478	1945.7241
X Variable 1	20689683.8784	21747918.0363
N-oxide	**ANOVA**	
	Degree of freedom (df)	**Sum of squares (SS)**
Regression	1	21732116399.7025
Residual	5	5734902.0117
Total	6	21737851301.7143
N-oxide	**Confidence Intervals**	
	Lower 95%	**Upper 95%**
Intercept	-1067.2941	2317.3851
X Variable 1	32726428.5752	33972015.7453
Pioglitazone	**ANOVA**	
	Degree of freedom (df)	**Sum of squares (SS)**
Regression	1	11154571681.1543
Residual	5	3631851.7028
Total	6	11158203532.8571
Pioglitazone	**Confidence Intervals**	
	Lower 95%	**Upper 95%**
Intercept	-959.5021	1746.6455
X Variable 1	23215615.6658	24199168.7775

ANOVA: Analysis of variance; Regression data shows that the validated method was statistically proven.

Accuracy

The accuracy of an analytical procedure: expresses the nearness of treaty amongst the value which is accepted either as a conventional factual value or a recognized reference value and the significance found. For the quantitative approaches, at least nine determinations across the specified range should be obtained [26]. The accuracy percentage for PGR-II, PIO-II and N-oxide were 98-105% for drug substance from LOQ to 250.0% level. This result indicates that the method was accurate and appropriate as the mean accuracy value was within the limit (80-120%).

Specificity

Specificity is the capability to judge the compound of interest unequivocally in the presence of components, which may be anticipated to be existent. Typically, these might include impurities, degradants, matrix, etc. [26]. Specificity of the method was

demonstrated by the peak purity, (i.e. the purity angle is lesser than the purity threshold) by way of the diode array detector for degraded samples. The specificity of the method was established with the pioglitazone in the existence of related impurities namely PGR-II, PIO-II and N-oxide. To prove the specificity; all forced degraded studied samples were performed at a test concentration of 2000 µg/ml. The peak purity analysis was homogenous for PGR-II, PIO-II and N-oxide. There was no interference observed from blank peaks and impurities**.** There was no secondary peak aroused from degraded samples. The results of forced degradation study indicate that the method was stability indicating. The impurity PGR-II and PIO-II were process related impurities and N-oxide was degradation impurity.

Robustness

By careful variation in chromatographic conditions, the resolution between PGR-II, PIO-II, N-oxide and pioglitazone were evaluated. The mobile phase flow rate was 1.0 ml/min. To check the effect of flow rate on the resolution, 0.1 units changed it from 0.9 to 1.1 ml/min. The column oven temperature was 45 °C. To check the effect of temperature on the resolution, 5 units changed it from 40 °C to 50 °C. The % of mobile phase-A composition was 80%. To check the effect of mobile phase composition on the resolution, 2% units changed it from 78% to 82%. The % of mobile phase-B composition was 20%. To check the effect of mobile phase composition on the resolution, 2% units changed it from 18% to 22%. The resolution between impurities and pioglitazone was greater than 5 in all the varied chromatographic conditions carried out (flow rate, the addition of trifluoroacetic acid and column temperature). The result shows that the method was considered robust.

Solution stability

The solution stability of pioglitazone and its impurities was carried out by freshly prepared standard and sample solution in a tightly closed volumetric flask at the room temperature (22-27 °C) as well as in the refrigerator at 2-8 °C for initial, 24 and 48 h. The result of solution stability shows that the solution was stable up to 48 h at room temperature (22-27 °C) and also at 2-8 °C in the refrigerator.

DISCUSSION

The USP resolution between PIO-II and pioglitazone was 15.9 which were 5 times more than that the obtained value of the research article [27]. The system precision results were very well within the acceptance criteria, i.e., %RSD for PIO-II, PGR-II, N-oxide and pioglitazone were observed within 0.5%. The %RSD value was obtained by the research work [27] was above 0.5% when compared to the present research study which was observed less than 0.5%. Moreover, the %RSD obtained in the validation was very less when compared to the previously published articles. The limit of detection and limit of quantitation results indicated that the method was sensitive to determine the content PGR-II, PIO-II and N-oxide in the sample of pioglitazone drug substance.

The method precision results show that the developed method was very precise with the addition of impurities in the presence of pioglitazone hydrochloride when compared to the previously established works. The detection and quantitation limits of PIO-II, PGR-II, N-oxide and pioglitazone were observed low when compared to the previously published article [27] though the study was performed for several impurities. The developed method was linear from very low level to high level when compared to the previously published article [27]. The accuracy of the method has been demonstrated by the presence of impurities in pioglitazone hydrochloride at the specified levels with respect to the test concentration; the results show that the method was very accurate at the LOQ level itself. The specificity, solution stability and robustness of the method show that the impurity PGR-II and PIO-II were process related impurities and N-oxide was degradation impurity. This indicates that the equipment was suitable, accurate, precise, sensitive and fit for study.

CONCLUSION

The developed RP-HPLC method was developed and validated as per ICH guidelines in terms of system suitability, system precision, method precision, specificity/stress studies, accuracy, linearity, robustness, solution stability, limit of detection and limit of quantitation for the quantitative estimation of related substances of pioglitazone hydrochloride drug substance. The correlation coefficients were greater than 0.99. The precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries after spiking experiments were between 98 and 105%, indicative of accurate method. Degradation studies reveal that the developed method was stability indicating hence, this method can easily and conveniently adopt for routine quality control analysis of the determination of related substances of pioglitazone drug substances in quality control laboratories.

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CONFLICTS OF INTERESTS

There is no conflict of interest to declare

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