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Separation and quantification of impurities of pioglitazone hydrochloride from active harmaceutical ingredient (API) using RPLC

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ABSTRACT

High performance reverse phase liquid chromatography (HPLC) method was developed for quantification of impurities of pioglitazone hydrochloride from active pharmaceutical ingredient (API). Pioglitazone hydrochloride and its impurities were separated using reverse phase gradient HPLC method. The experimental procedure involved variable wavelength detector (at 230nm), the mobile phases consisting of mobile phase A of phosphate buffer and mobile phase B of acetonitrile and methanol mixture (65:35) were used. The flow rate of the mobile phase was adjusted to 1.0mL per minute on an Inertsil ODS 3V, C-18 (250mm × 4.6mm, 5µ size) column. A calibration curve showed good linearity within the LOQ to 0.4% of the test range (ie 0.2 ppm to 4 ppm). The recoveries ranged from 93% to 105%. The % RSD for repeatability was 2.5. The HPLC method is capable of detecting all process related impurities, which may be present in the API. The method was validated and from the results obtained it can be concluded that the method is suitable for the intended purpose of separation and quantification.

KEYWORDS

HPLC;
Pioglitazone hydrochloride;
Active pharmaceutical ingredient (API);
Impurity (I- IV).

INTRODUCTION

Pioglitazone hydrochloride (RS)-5-{4-[2-(5-ethyl pyridin-2yl) ethoxy] benzyl}-2-4 thiozolidinedione hydrochloride is an anti-diabetic drug (thiazolidinedione-type, also called "glitazones") used along with a proper diet and exercise regime to control high blood sugar in patients with type 2 diabetes (non-insulin-dependent diabetes). It works by helping to restore the body's proper response to insulin, thereby lowering blood sugar. Effectively controlling high blood sugar helps prevent heart disease, strokes, kidney disease, blindness, blood circulation problems as well as sexual impairment problems (impotence). The product is developed by Takeda chemicals[1-2].

Although several methods including high performance liquid chromatography, Spectrophotometry and TLC etc are available for determination of impurities from pioglitazone hydrochloride Formulation and API [3-8], no reference is found for simultaneous determination of 5-{4-[2-(5-Ethyl pyridin -2yl) propoxy] benzyl}-2,4-thiazolidinedione (Impurity I) and 5,5'(4,4'-(2-(5-ethyl pyridin-2-yl)propane-1,3-diyl) bis(oxy) bis(4,1-phenylene) bis(methylene) dithiazolidine-2,4-dione (Impurity II) along with 5-Ethyl-2-(2-(4-bromophenoxy) ethyl) pyridin (Impurity III) and 5-{4-[2-(5-Ethyl pyridin-2-yl) ethoxy] benzyl}-3-{4-[2(5-ethyl-pyridin-2-yl) ethoxy] phenyl} -1,3 thiazolidine -2,4 dione (Impurity IV).

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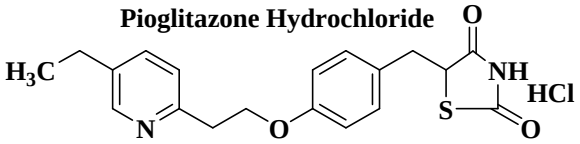
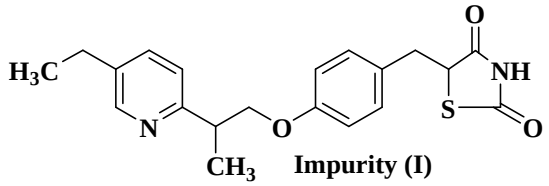
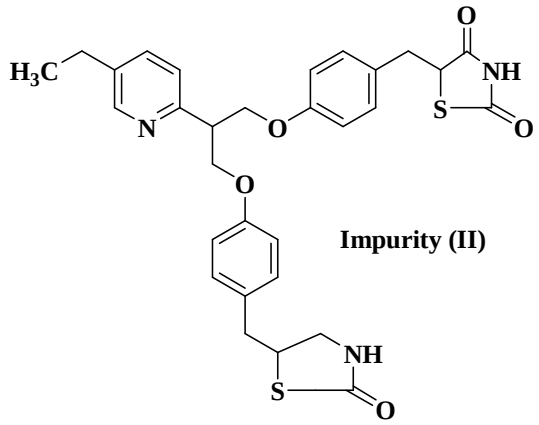
Structure of pioglitazone hydrochloride and related impurities



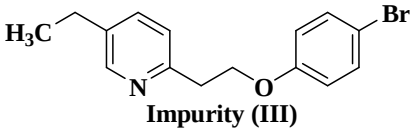
5-{4-[2-(5-Ethyl pyridin-2-yl) ethoxy] benzyl}-2,4 thiazolidine dione hydrochloride



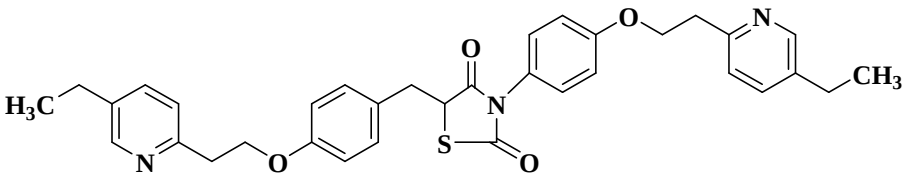
5-{4-[2-(5-Ethyl pyridin-2yl) propoxy] benzyl}-2,4-thiazolidinedione



5,5'(4,4'-(2-(5-ethyl pyridin-2-yl)propane-1,3-diyl) bis(oxy) bis (4,1-phenylene) bis(methylene) dithiazolidine-2,4-dione



5-Ethyl-2-(2-(4-bromophenoxy) ethyl) pyridin



5-{4-[2-(5-Ethyl pyridin-2-yl) ethoxy] benzyl}-3-{4-[2(5-ethyl-pyridin-2-yl) ethoxy] phenyl} -1,3 thiazolidine -2,4 dione

The force degradation study showed degradation of pioglitazone hydrochloride in alkaline conditions. Impurities I and II are generated from the raw material, Impurities III and IV are generated during synthesis. The ratio of mobile phase A and mobile phase B was adjusted in such a way that degradents and above impurities were well separated from pioglitazone hydrochloride peak.

The proposed method is validated according to ICH guidelines Q2A and Q2B[9]. The method has been applied satisfactorily for the determination of impurities of pioglitazone hydrochloride in active pharmaceutical ingredient, with satisfactory results.

EXPERIMENTAL

Instrumentation

A liquid chromatography system consisted of HP 1100 series equipped with degasser, a quaternary solvent delivery pump, autosampler, column thermostat and photodiode array detector. The system was controlled by chemstation B.03.01 software. For chromatographic analysis an Inertsil ODS 3V, C-18 column (250mm × 4.6mm, 5µ size) was used.

Materials used

Standard pioglitazone hydrochloride and impurities from Invent Farma Pvt. Ltd., acetonitrile, methanol and triethylamine were of HPLC grade from Qualigens; potassium hydrogen phosphate and orthophosphoric acid were of analytical grade from Merck and HPLC grade water purified by Mill Q Academic (USA).

Procedure

Chromatographic conditions

Column	: Inertsil ODS 3V C-18, (250mm × 4.6mm, 5µ size)
Flow rate	: 1.0 mL per minute
Column Oven temperature	: 25^{0}C
Detector	: UV at 230nm.
Injection volume	: 10 µL
Run time	: 45 minutes
Mobile Phase A	: Potassium phosphate buffer
Mobile Phase B	: Acetonitrile : Methanol(65:35)

Gradient program

Time (min.)	Mobile phase A%	Mobile phase B%
0	40	60
10.0	40	60
20.0	25	75
35.0	25	75
40.0	40	60
45.0	40	60

Preparation of buffer

1.36g of potassium dihydrogen phosphate was accurately weighed and transferred to a 1000 mL standard volumetric flask. About 800 mL of purified water and 1mL of triethylamine were added, the pH was adjusted to 6 ±0.05 with dilute orthophosphoric acid and diluted upto the mark with HPLC grade water and filtered through 0.45µ, Ultipore N_{66} Nylon 6,6 membrane, degassed in ultrasonic bath.

Validation of method[9-11]

Preparation of system suitability

The system suitability experiment was carried out by injecting the mixture of all impurities and pioglitazone hydrochloride into the chromatographic system (1000ppm of pioglitazone hydrochloride and 2 ppm of all impurities). Theoretical plates (N), Tailing factor (T) and Resolution(R) are listed in TABLE 1.

Specificity

To demonstrate the specificity of the method, diluent was injected into the chromatographic system and checked for interference at retention time corresponding to the retention of pioglitazone hydrochloride and its impurities (methanol was used as diluent for preparation of standard and sample solution). Accelerated degradation studies were also carried out to demonstrate the validity of the method. The sample was refluxed with 0.1N HCl. Exposure to temperature and UV light did not give any degradation impurities but the sample exposed to 0.1N NaOH completely decomposed to give degradation products. The sample after exposure to 0.1N HCl, 0.1N NaOH, UV, oxidation and heat was checked for the peak purity of pioglitazone hydrochloride by photodiode array detector. It is concluded that pioglitazone hydrochloride peak had no detectable impurity embedded in and was free of co-eluting degradation compounds. The diluent and degraded compound does not interfere with the pioglitazone hydrochloride and impurity peaks (Figures 1-4).

Limit of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined using Standard deviation of the response and slope of the calibration curve technique (TABLE 2).

Linearity of detector response

The linear working range was selected depending upon the nature of application. In this experiment the

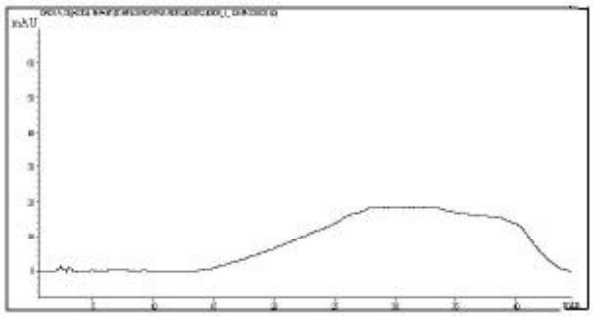


Figure 1 : Typical chromatogram of blank

TABLE 1 : Data of pioglitazone hydrochloride (System suitability)

Parameters	Pioglitazone Hydrochloride (RT -9.547)	Impurity I (RT -13.624) (RRT -1.43)	Impurity II (RT -15.190) (RRT -1.59)	Impurity III (RT -25.487) (RRT -2.67)	Impurity IV (RT -31.190) (RRT -3.27)
Theoretical Plates	13056	14828	18434	75875	49487
Peak symmetry	0.862	1.052	1.075	0.998	1.038
Resolution	-	10.413	3.495	25.146	12.233

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TABLE 2: Statistical data of pioglitazone hydrochloride (LOD, LOQ and Linearity)

Parameters	Pioglitazone hydrochloride	Impurity I	Impurity II	Impurity III	Impurity IV
LOD (µg/mL)	0.015	0.12	0.14	0.07	0.11
LOQ (µg/mL)	0.05	0.39	0.46	0.24	0.35
Range (µg/mL)	0.2 to 0.4	0.189 to 3.785	0.188 to3.765	0.21 to 4.21	0.215 to 4.306
Slope	30.421	22.345	20.815	32.604	26.503
% Intercept	0.3148	1.1411	0.6319	1.0182	2.031
Correlation coefficient(r)	1.000	0.9995	0.9992	0.9998	0.9997

TABLE 3 : Statistical data of pioglitazone hydrochloride (Recovery)

Parameters	Impurity I	Impurity II	Impurity III	Impurity IV
%R.S.D. of replicate standard	1.06	0.92	1.11	1.26
%Recovery at three levels	96.41 -102.96	93.34 -100.90	96.86 -104.87	95.61 -104.39

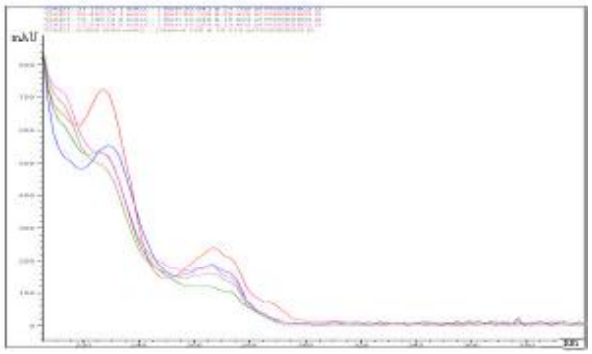

Figure 2 : UV Spectrum of pioglitazone hydrochloride and impurities

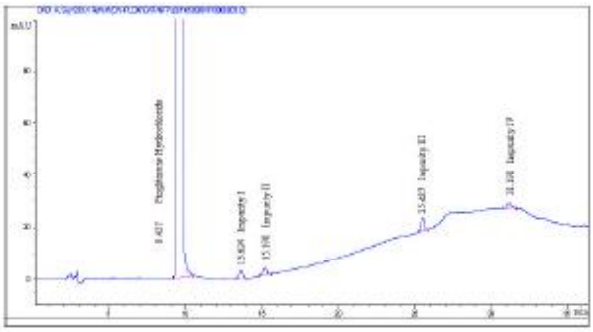

Figure 3 : Typical chromatogram of system suitability solution

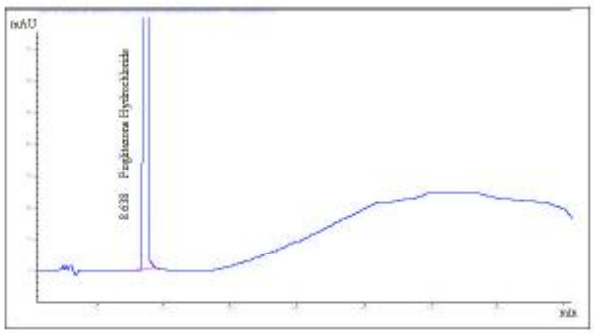

Figure 4 : Typical chromatogram of as such sample

method was applied for the determination of impurities and hence detector response was checked from LOQ to 0.4% of the working concentration of the pioglitazone hydrochloride. For present study, the working concentration of pioglitazone hydrochloride was 1 mg/mL. Seven levels were prepared and each level was injected in duplicate into the chromatographic system. Mean peak area of each level was calculated. Graph of mean peak area vs. concentration was plotted and the best-fit line was determined by linear regression. % Intercept and Correlation coefficient (r) obtained are given in TABLE 2.

Recovery studies

The experiment was carried out at four different levels i.e. LOQ, 0.1%, 0.2% and 0.4% of the concentration of pioglitazone hydrochloride (1mg/mL). The impurity standard at these four levels was added to the active pharmaceutical ingredient. From the amount found, the % recovery was calculated. The results obtained are listed in TABLE 3.

Precision repeatability I

Accurately weighed 1gm of pioglitazone hydrochloride was taken in a round bottom flask and 20 ml each of stock solution of impurities prepared in methanol (0.1mg/mL) were added, and the mixture was sonicated to dissolve the sample. Methanol was evaporated by vacuum distillation. The sample was dried under vacuum at 60^{0}C. About 50 mg of dried sample was weighed accurately and transferred to a 50 mL standard volumetric flask. 25 mL of methanol was added. The mixture was sonicated for 10 minutes for dissolution, diluted up to the mark with methanol and mixed.

TABLE 4: Statistical data of pioglitazone hydrochloride (Precision Repeatability I, II and solution stability)

Parameters	Impurity I	Impurity II	Impurity III	Impurity IV
%RSD of precision repeatability I	1.21	1.91	2.18	1.91
%RSD of precision repeatability II	0.92	1.28	0.91	1.98
%RSD of precision repeatability I and II	1.26	1.55	1.60	2.11
Solution stability (Ratio)	1.01	1.00	1.003	1.020
RRF	0.735	0.684	1.071	0.871

The solution was then filtered through 0.45µ Ultipore N_{66} Nylon 6,6 membrane and injected into the chromatographic system. The amount of each impurity was calculated against the impurity standard and also with pioglitazone hydrochloride low load using RRF. The RRF is ratio of peak response per unit concentration for each impurity to the peak response per unit concentration for the reference compound (pioglitazone hydrochloride). This procedure was repeated six times weighing the sample individually each time. The % RSD for repeatability of each impurity and RRF values are listed in TABLE 4.

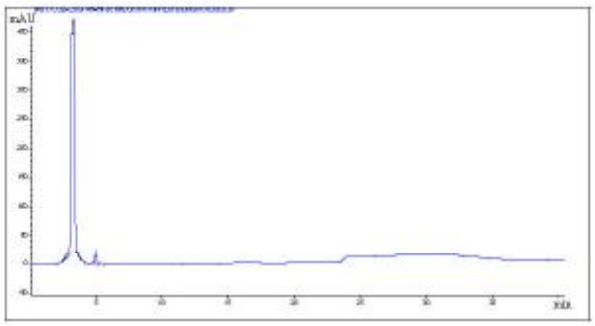

Figure 5 : Typical chromatogram of degraded sample in 0.1NaOH

Precision repeatability II

Same procedure was followed as per Precision repeatability I on the next day using the same system. Sample and standard were freshly prepared.

% RSD of Precision Repeatability I and Precision Repeatability II was found to be less than 2.5 (TABLE 4).

Solution stability

Solution stability of precision repeatability I sample was checked for 24 hours at room temperature by comparing the assay values after 24 hours with the initial assay value against the fresh standard. Ratio of the results of samples after 24 hours to that of initial results were within the range of 1 ± 0.5 which indicate satisfactory solution stability (TABLE 4).

Optimisation of parameters

Selection of detection conditions

Aliquot of 100ppm concentration was injected into the chromatographic system. Using diode array detector, the standard solution was scanned from 200nm to 400nm. Two maximae were seen and the maximum absorption was observed at 230nm.

Optimisation of extraction conditions

The extraction conditions including extraction method, extraction solvent and extraction time were tested. As pioglitazone hydrochloride is practically insoluble in water and sparingly soluble in methanol and acetonitrile, methanol was selected as extraction solvent.

Optimisation of separation conditions

The chromatographic conditions were optimized to obtain good resolution, tailing factor and theoretical plates within shortest possible analysis time. Various buffers and mobile phase ratios were tried out to separate all the impurities and the retention time was also checked using a gradient program. Influence of mobile phase pH in the range of 2.5 to 6 on resolution of impurities was checked. It was observed that there was no impact of mobile phase pH on the resolution. Different types of columns such as Peerless C-18, Zorbax C-18 and Inertsil ODS 3V C-18 were tried out. Inertsil ODS 3V C-18 was found to be most suitable.

RESULTS AND DISCUSSION

For interday precision and intraday precision % RSD was less than 2.5% for all impurities, which shows high precision. Percentage recovery in between 93% to 105% from LOQ level to 0.4% level indicates specificity and accuracy of the method.

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CONCLUSION

The HPLC method has been developed for quantitative determination of pioglitazone hydrochloride impurities in active pharmaceutical ingredient. The developed HPLC method was found to be specific, sensitive, accurate and precise. Hence the method can be used for routine determination of impurities in active pharmaceutical ingredient.

ACKNOWLEDGMENTS

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