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ARTICLES

Validated Reversed Phase HPLC Method for Determination of Pioglitazone Hydrochloride in Bulk Drug and Tablet Formulations[1]

Prem Sunder Prasad, Syed Sarim Imam, Mohammed Aqil*, Mohammad Rizwan, Yasmin Sultana, and Asgar Ali

Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University M. B. Road New Delhi 110062, India

**e-mail: aqilmalik@yahoo.com*

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Abstract—An accurate, sensitive and precise HPLC method was developed and validated for the routine analysis of pioglitazone hydrochloride in dosage forms. The analyte was chromatographed on a C-18 column using a mixture of acetonitrile and ammonium acetate buffer (pH 5.0) as mobile phase in ratio 60 : 40 (v/v) at flow rate 1.0 mL/min. Linearity range was found to be 10–100 μg/mL with a correlation coefficient r^2 = 0.9984. The method was validated for precision, accuracy, robustness, specificity and sensitivity, using bulk drug samples. Application of method in assay of bulk drug and tablets revealed mean recoveries range from 99.88–100.32%. Due to its simplicity, rapidity, high precision and accuracy, the proposed method may be used for determining pioglitazone hydrochloride in bulk and dosage forms.

Keywords: pioglitazone, high performance liquid chromatography, tablet formulations

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Pioglitazone hydrochloride (**PGT**), a thiazolidinedione class anti-diabetic agent, is chemically [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]-methyl]-2,4-] thiazolidine- dione monohydrochloride [1, 2]. Various analytical methods such as UV-spectrophotometry [3, 4], liquid chromatography and micellar electrokinetic chromatography [5–9], high performance liquid chromatography [10–14] were reported for estimation of PGT in various matrices [15, 16]. These reports revealed complex processing and costly equipment. However, no routine method has been reported for determination of PGT in different dosage forms including transdermal therapeutic system.

EXPERIMENTAL

Instrumentation. HPLC apparatus (Agilent Technologies 1120 compact LC system) equipped with a model LC-20AT pump and SPD 20A variable wavelength detector and a Rheodyne injection valve with a 20 μL loop was used for development and evaluation of this method. A Hypersil ODS C-18 (250 × 4.6 mm, 5 μm particle size) was used as separating column. Data processing was performed with Agilent LC Ezchrom software.

Drug and chemicals. PGT was obtained from Ranbaxy laboratories (Gurgaon, Haryana, India) and marketed drug product Piosys (ONTC Pharmaceuticals Ltd., Bangalore, India). Pioz (USV. Ltd., Himachal Pradesh, India) was procured from a community pharmacy. All other reagents were of HPLC grade. HPLC grade water was prepared using a Milli-Q system (Millipore, India).

Method development and validation. The mobile phase used in the study was a mixture of acetonitrile-ammonium acetate buffer (pH 5) 60 : 40 (v/v). The mobile phase was filtered through a 0.45 μm membrane and degassed by sonication for 15 min before use. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 mL/min and the volume of the analyte sample injected was 20 μL. The eluents were monitored at 270 nm using UV-visible detector. All the chromatographic separations were performed at 25 ± 1°C.

Method validation. The above prepared method was validated as per International Conference on Harmonization (**ICH**) guidelines [17]. The linearity of the method was determined by diluting the standard stock solution to 10–100 μg/mL. The dilutions were analyzed and peak area response against concentration. Calculated precision was assessed by measurement of repeatability (intra-day) and intermediate (inter-day) precision, in accordance with ICH guidelines. Repeatability was studied by assay of six different concentrations of the drug (10, 30 and 50 μg/mL) in one laboratory by the same analyst on one working day. Intermediate precision was checked by repeating the studies on three different days.

[1] The article is published in the original.

The accuracy of the method was determined as recovery from 25 μg/mL standard solution spiked with 50, 100, and 150% extra PGT. Method robustness was assessed by deliberately changing mobile phase flow rate and proportion of acetonitrile to 1.0 ± 0.2 mL/min and 60 ± 2% (v/v), respectively.

Limits of detection (**LOD**) and quantification (**LOQ**) were determined by the standard deviation (*s*) method [10]. Blank samples (i.e., drug-free samples) were injected in triplicate and peak area was recorded. LOD and LOQ were determined from the slope (S_l) of the linearity plot and the s of the response to the blank sample by use of the formulae LOD = $3.3s/S_l$ and LOQ = $10s/S_l$.

Assay of PGT in bulk samples and tablets. Ten tablets were weighed to get the average weight and pulverized. Powder equivalent to 10 mg PGT was extracted with methanol and sonicated for about 15 min and made-up the volume to 10 mL to get a stock solution of 1 mg/mL. The solution was filtered through 0.45 μm membrane filter. The solution was further diluted with mobile phase to get 20 μg/mL with methanol and analyzed by the above liquid chromatography method and the drug content in the tablets was quantified.

RESULTS AND DISCUSSION

Method development. HPLC method with UV detection was developed for the determination of PGT. A number of mobile phases were initially attempted to elute the analyte. The main focus was to achieve sharp and Gaussian shaped peaks. Therefore, acetonitrile and water in different proportions were used but no sharp peak was observed. Ammonium acetate was then added to increase the polarity of the mobile phase. The suitable mobile phase composition was found to be 20 mM ammonium acetate at pH 5 and acetonitrile in the ratio of 40 : 60 (v/v). A sharp and isolated peak was obtained at retention time 5.24 min. The applied chromatographic conditions permitted a good resolution of with no drug decomposition observed during the analysis.

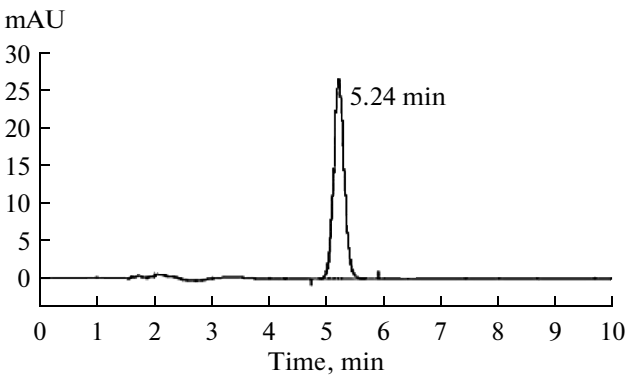
Method validation. A linear response ($r^2 > 0.9984$) was obtained as function of concentration in the range 10–100 μg/mL. The regression equation for the calibration plot was $y = 296736x + 324271$ ($n = 3$, detection at 270 nm). No significant difference between the slopes of calibration plots was observed on different days (*t*-test, $p > 0.05$). The method was precise, as relative standard deviation (**RSD**) of repeatability and intermediate precision were in the range 0.26–0.87 and 0.27–0.94%, respectively. The accuracy, calculated as percentage recovery was 99.88–100.32%. Low values of the RSD in the range of 0.13–0.31% and good percentage recovery were indicative of the excellent accuracy of the method. Student's t-test showed no significant difference ($p > 0.05$) between results from study of accuracy and the theoretical concentration. On deliberate change in mobile composition and flow rate, no significant ($p > 0.05$) in area and retention time of standard solution (25 μg/mL) was achieved. Low RSD values in the range 0.582–1.05% for these results substantiate the consistency of the method. LOD and LOQ were 0.332 and 0.996 μg/mL, respectively, indicating the sensitivity of method.

Results (mg/tablet) of analysis pioglitazone hydrochloride in tablets dosage form ($n = 5$)

Drug	Claimed	Found	Mean recovery, %	RSD, %
Pioz®	15	15.1 ± 1.1	100.8	7.8
Piosys®	15	15.0 ± 1.1	100.5	7.3

Assay of PGT in bulk and tablets. Assay of marketed product of PGT like *Piosys* and *Pioz* tablets showed mean recovery 100.53 and 100.86%, respectively (table). Chromatogram of PGT shown in the Figure revealed that none of the tablet ingredients interfered with the analyte peak. Retention time of chromatogram of bulk drug and tablet formulation showed insignificant change (5.24–5.97 min, $p > 0.05$) which confirm the selectivity of the method and no other peaks appeared with the analyte.

The proposed method was found to be simple, precise, accurate, selective, and rapid for determination of PGT in bulk samples and tablets. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims which suggested non interference of formulation excipients in the estimation.



A typical HPLC chromatogram of pioglitazone hydrochloride in tablet dosage form.

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