A new RP-HPLC method for separation and determination of process related impurities in Pioglitazone Hydrochloride API

D Srinivasulu*, B S Sastry, G O Prakash and D N S S Archana
University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh 530003, India

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The objective of the study was to develop a validated, specific and stability-indicating gradient reverse phase liquid chromatographic method for determination of Pioglitazone Hydrochloride along with its impurities in bulk samples. Drug substance was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolytic, humidity and thermal degradation as per International Conference on Harmonization (ICH) to show the stability-indicating power of the method. Significant degradation was observed with alkali and hydrogen peroxide. The impurities were characterized using spectral techniques like IR, $^1$H NMR and MS. Successful separation of impurities was achieved on C18 ODS (150x4.6 mm) 3.5 µ column using mobile phase consisting of Solvent A: Ammonium acetate buffer and Acetonitrile in the ratio (57:43 v/v) for 0-7 min and Solvent B: Ammonium acetate buffer and Acetonitrile in the ratio (20:80 v/v) at a flow rate of 1.0 ml/min from 7-20 min followed by Solvent A from 20-21 min. The retention times of impurity A, impurity B, impurity C and Pioglitazone were 3.44, 10.65, 17.95 and 8.32 min respectively. The detection wavelength was set at 254 nm with column temperature at 45 ºC. Finally developed method was validated as per ICH guidelines for specificity, linearity, precision and accuracy.

Keywords: Stability Indicating, Liquid Chromatography, Pioglitazone Hydrochloride, Validation.

Introduction

Pioglitazone hydrochloride is an anti diabetic agent which is used in the management of type 2 diabetes mellitus also known as Non-Insulin-Dependent Diabetes Mellitus [NIDDM] or adult-onset diabetes. It acts by stimulating nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-gamma) which regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport and utilization and there by improves insulin sensitivity. It is chemically designated as [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2, 4-] thiazolidinedione monohydrochloride and its empirical formula is C$_{19}$H$_{20}$N$_{2}$O$_{3}$S•HCl, having a molecular weight of 392.90 daltons. The following analytical methods have been reported for determination of Pioglitazone hydrochloride and its impurities in dosage forms as well as biological fluids. Lofty saber reported a HPLC method for determination of Pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC (Micellar electrokinetic chromatographic) methods. Yamashita et al., have developed a HPLC method for determination of Pioglitazone and its metabolites in human serum and urine. Analytical methods for the determination of Pioglitazone and its metabolites in human serum have been reported using HPLC/UV by Zhong and Williams and HPLC/MS by Xue et al.,. Tahmasebi et al., have studied an extraction of trace amounts of Pioglitazone with hollow fiber liquid phase micro extraction by HPLC-UV detection in biological fluids. Sripalakit et al., have reported a HPLC method for the determination of Pioglitazone in human plasma using UV detection and its application to a pharmacokinetic study. Smita et al., studied degradation behavior of Pioglitazone hydrochloride in bulk and pharmaceutical formulations using HPLC. Rashmitha et al., developed a stability indicating HPLC method for the determination of impurities in Pioglitazone hydrochloride. Narayan et al., Separated and quantified impurities of Pioglitazone hydrochloride from active pharmaceutical ingredient (API) using RPLC. Pawar et al., reported an Impurity profile study of Pioglitazone and Glimepiride combination by liquid chromatography. Gurusamy et al., developed RP-HPLC Method for Simultaneous Estimation of Pioglitazone, Glibenclamide and Glibenclamide Impurities in a Combination Drug Product.
The present study is aimed at separation and determination of Pioglitazone hydrochloride and its process-related impurities by RP-HPLC method. The developed method was thoroughly validated as per ICH guidelines and subjected to forced degradation studies on stability indicating factors for acid and base hydrolysis, oxidation, thermal, photolytic and humidity conditions.

**Experimental Section**

**Materials**
Pioglitazone hydrochloride working standard and its process related impurities Impurity-B (5-{4-[2-(5-Ethyl-2-pyridinyl) ethoxy] benzylidene}-2,4-thiazolidinedione), Impurity-C (5-[4-(2-(5-Ethylpyridin-2-yl) ethoxy) benzyl]-3-(2-(5-ethylpyridin-2-yl) ethyl) thiazolidine-2, 4-dione) were synthesized at Pharmazell Research Centre, Visakhapatnam (India) and obtained as gift samples. HPLC grade Acetonitrile was obtained from Merck. Analytical grade Ammonium acetate and acetic acid was used (Rankem, India). High purity water was prepared by using Milli-Q academic purification system (Mllipore). 5-(4-(2-(5-Ethylpyridin-2-yl)ethoxy)benzyl)-2-iminothiazolidin-4-one (Impurity A) was purchased from SL Drugs & Pharmaceuticals, India. The impurities were characterized using spectral techniques like IR, 1H NMR and MS (Table 1).

**Instrumentation**
The LC system used for method development, forced degradation studies and method validation consisted of Waters alliance system with 2695 model pump with 2998 with photodiode array detector and 2695 model pump with 2489 UV detector (Mileford,USA). The output signal was monitored and processed using empower software. Photo stability studies were carried out in a photo stability chamber. Thermal stability studies were carried out in a dry air oven.

**Chromatographic conditions**
Inertsil ODS C18 analytical column (150 mm × 4.6 mm, 3.5µ) was used for the analysis at 45 °C. The mobile phase was pumped through the column at a flow rate 1.0 ml/min. The sample injection volume was 20 µl. The UV detector was set to a wavelength of 254 nm for the detection.

**Solutions**

**Mobile phase**
A mixture of aqueous 0.02 mol/l Ammonium Acetate (pH of the buffer adjusted to 4.6±0.05 with acetic acid, filtered and degassed in an ultrasonic bath) and Acetonitrile was used as mobile phase in the ratio 57:43 v/v (solvent A) and 20:80 v/v (solvent B).

_Diluent:_ Tetrahydrofuran and Methanol were prepared in 1:3 ratio and used as diluent.

**Impurity mixture**
38 µg/ml solution of Pioglitazone hydrochloride and its process related impurities (Impurity A, B and C) was prepared using diluent. This impurity stock solution was adequately diluted to study accuracy, precision, linearity, robustness, limit of detection and quantitation.

**Standard solution**
1 mg/ml solution of Pioglitazone hydrochloride, working standard, was prepared using diluent and injected into the system.

**Sample solution**
1 mg/ml solution of Pioglitazone hydrochloride sample was prepared using diluent and injected into the system.

**Forced degradation samples for specificity study**
The stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation and UV degradation. Pioglitazone was heated with aqueous 0.1 N HCL solution at 80 °C for 24 hrs and separately with aqueous 0.1 N NaOH at 80 °C for 24 hrs to study formation of degradation products under acidic and basic conditions. Drug substance was heated with 3% hydrogen peroxide solution at 80 °C for 24 hrs to study formation of degradation products under oxidative condition. It was exposed to UV light at 254 nm for 24 hrs and sample was kept at room temperature and at 105 °C for 24 hrs to study formation of degradation products under humidity and thermal conditions respectively.

**Results and Discussion**

**Method development**
The main objective of the chromatographic method was to develop a suitable and robust RP-HPLC method for the determination of Pioglitazone hydrochloride and its process related impurities by using different mobile phases and columns to achieve the best separation and resolution. The method development was initiated with C18 column using a mobile phase containing water and Acetonitrile as organic modifier. Broad peaks were observed with this mobile phase hence in the above conditions mobile phase was replaced with phosphate buffer but
peak symmetry and resolution between impurities was very poor. To improve the peak shapes, ammonium acetate buffer was used instead of phosphate buffer. Though it resulted in sharp peaks, the impurities were merging. Hence a gradient elution mode was tried where many experiments were conducted by using different gradient programs and columns while optimizing the pH of buffer, buffer concentration, organic modifier strength and wavelength. Finally the best results were observed using a column C18 ODS (150x4.6 mm), mobile phase consisting of Solvent A: ammonium acetate buffer and Acetonitrile in the ratio (57:43 v/v) and Solvent B: ammonium acetate buffer and Acetonitrile in the ratio (20:80 v/v) at a flow rate of 1.0 ml/min. According to gradient elution program 100% solvent A was used for 0-7 min, 100% solvent B for 7-20 min and 100% solvent A for 20-21 min. The above chromatographic conditions resulted in sharp peaks with minimum tailing, good resolution and shorter runtime for Pioglitazone hydrochloride and its process related impurities. The retention times of impurity A, impurity B, impurity C and Pioglitazone were 3.4, 10.65, 18.0 and 8.4 min respectively. The developed LC method was determined to be specific for Pioglitazone hydrochloride and its process related impurities.

Method Validation

The proposed method was validated per ICH guidelines\textsuperscript{14}. Specificity

The chromatogram of drug with impurities was compared with the blank chromatogram to verify the blank interference. No peak was observed at the retention time of Pioglitazone hydrochloride and its impurities. Hence the method is specific for the determination of process related impurities in Pioglitazone hydrochloride.

Linearity

The linear calibration plot for Pioglitazone and its impurities was determined over the calibration ranges (LOQ (limit of quantification) to 150% Specification level, a correlation coefficient of greater than 0.99 was obtained. The results were reported (Table 2) and showed an excellent correlation between the peak areas and concentrations of Pioglitazone and its impurities.

Precision

The %R.S.D of Pioglitazone was found to be less than 2.0% (R.S.D). Repeatability and intermediate precision for the process-related impurities in Pioglitazone hydrochloride were found to be less than 1.0% R.S.D revealing the high precision of the method (Table 2).

Accuracy/Recovery

The percentage recoveries of impurities in the drug substance ranged from 94.7-109.2 which is within the limit. This indicated that the method is accurate to determine the process impurities in Pioglitazone hydrochloride. The mean recoveries of all the impurities were calculated (Table 2).

Results of forced degradation studies

Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation path ways of drug as well as interaction between the drug and the excipients in drug product. Knowledge from stability studies enables understanding of the long-term effects of the environment on the drugs. The above study indicates that there is no degradation at room temperature, photolytic, thermal and acid hydrolysis. Pioglitazone hydrochloride shows significant degradation only with alkali and hydrogen peroxide and the degraded peaks are well separated from the main peak (Table 3). The separation, peak purity results showed that the assay method is specific and capable of picking up all the degradation peaks. Hence it was concluded that the method was very selective and stability indicative and suitable for the determination of impurities in Pioglitazone hydrochloride API.

Conclusion

A new gradient RP-HPLC method was developed for the separation and determination of process related impurities in Pioglitazone hydrochloride and validated as per ICH guidelines. The method was found to be simple, selective, precise, sensitive, robust, accurate with shorter run time. Therefore, this method can be used for routine testing as well as stability analysis of Pioglitazone hydrochloride drug substance. All statistical results (Mean, %RSD and %recovery) were within the acceptance criteria.

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References