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Alkaline Hydrolysis of Pioglitazone Hydrochloride by Rp-Hplc: Stress Stability Study

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Abstract

HPLC method was developed to study alkaline degradation of pioglitazone HCl in 0.1N NaOH at 80°C. The analysis of reaction mixture showed that degradation started immediately, the primary degradation product was eluated at 2.4±0.1 minute having a resolution of 13.4±0.5 with pioglitazone HCl. As time was increased successively, area under the curve for primary degradant was increased and for pioglitazone HCl, it was decreased. After 120 minutes a secondary degradation product was observed having retention time of 8.2±0.1 minutes and degraded completely within 4 hours in 0.1N NaOH at 80°C.

Key-words- Reverse Phase High Performance Liquid Chromatography (RP-HPLC); Stress Stability; ICH Guidelines; Pioglitazone hydrochloride; Alkaline hydrolysis; Degradation.

Introduction

Pioglitazone HCl 5-(4-(2-(5-ethylpyridin-2-yl) ethoxy) benzyl) thiazolidine-2, 4-dione hydrochloride (fig.1), is an oral antihyperglycemic agent (Martindale, 2002) which is referred to as an "insulin sensitizer" because it is a potent activator and a highly selective agent for peroxisome proliferator- activated receptor gamma (PPARγ). It is used to improve glycemic control in patients with type -II diabetes (non-insulin-dependent diabetes mellitus). For determination of pioglitazone in human plasma, LC-MS/MS method and HPLC method has been reported (Jonlin, 2003; Yamashita, 2004; Radhakrishna, 2002; Zhong et al., 1996). But alkaline stability studies of pioglitazone is not well described in literature.

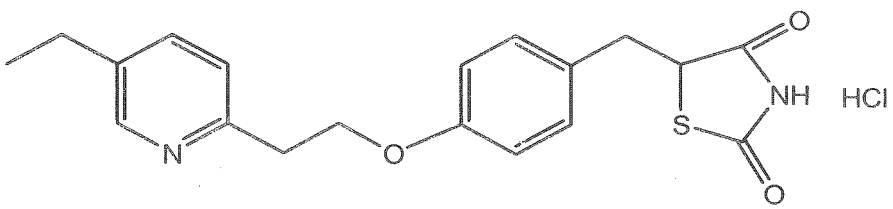

Figure 1. Chemical structure of pioglitazone hydrochloride

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The present work deals with the development of selective, accurate, precise and stability indicatory method with respect to alkaline hydrolysis for determination of pioglitazone HCl in the presence of its degradation products for assessment of purity of the bulk drug and stability of dosage form according to the ICH guidelines, 2002.

Material and Method

Material

Pioglitazone HCl reference standard was a generous gift of Aristo Pharmaceutical Ltd, Bhopal, India. Acetonitrile (HPLC grade) and all chemicals and reagents used were of analytical grade and were obtained from Merck Chemical India.

Instrumentation and chromatograph

HPLC analytical measurement and separation were performed using solvent delivery module LC-10ATvp Shimadzu Liquid chromatograph pump equipped with 20 µl loop, model SPD M10Avp Shimadzu UV/VIS diode array detector (λ= 226 nm) and Phenomenex, (250 x4.6 mm) Luna 5µ C 18 (2) 100 A Column. Mobile phase consisted of Acetonitrile : 10mM potassium dihydrogen phosphate buffer (pH -6), (50:50, v/v) was used and during analysis flow rate was maintained at 1.5ml/min and column temperature at 25-30°C. The sample was diluted with the water : acetonitrile (50:50, v/v).

Preparation of solution for standard calibration graph

To develop a suitable LC method for the determination of pioglitazone HCl and its degradations, standard of pioglitazone hydrochloride was accurately weighed and transferred to a 100.0ml volumetric flask, dissolved in 50.0ml of acetonitrile then volume was made up with triple distilled water (1000µg /ml, A). The accurate amount of stock solution (A) was taken and diluted to 100.0 ml with diluent to get working standard (100µg/ml, B). Aliquots of standard stock (B), solutions of 10, 20, 30, 40, 50, 60 and 70 µg/ml were prepared and subjected for analysis. Peak area under curve was observed and plotted against respective concentration and linearity was observed in the range of 10.0-70.0µg/ml. The solution of final concentration was stored at room temperature for 12.0 hours and analysed on HPLC every hour and all the system suitability parameters were optimized by freshly prepared standard solution.

Alkaline hydrolysis procedure

The accurately weighed pioglitazone HCl was dissolved in 0.1 NaOH and volume was made to get a solution of 1000 µg/ml with 0.1 NaOH. The above solution mixture was kept at temperature 80° C for 210 minutes. The aliquots were withdrawn at different time intervals, allowed to cool, neutralized with 0.1 N HCl and diluted with water : acetonitrile (50:50, v/v) to get a final concentration of 50 µg/ml. Prior injection, the solution was filtered through 0.45µ syringe filter, and were analysed for degradation pattern in aforementioned mobile phase. The peak area of pioglitazone HCl and degradation product were plotted against calibration curve.

Results and Discussion

Oral anti hyperglycemic agent pioglitazone HCl is less stable in alkaline medium. The hydrolysis of pioglitazone HCl was studied at three variable conditions, different concentration of sodium hydroxide, temperature and time (Singh, 2000; Yong, 1994; Andrzejewska et al., 2003) and it was found that degradation rate varied with the temperature and strength of alkali. All the system suitability parameters capacity factor, plate number, tailing factor, retention time, and resolution (with respect to degradation product peak) were optimized before starting the experiment and these parameter were found within the limit (Table 1).

Table 1. Results of System Suitability Parameter*

Retention time (min)	Capacity Factor	Plate Number	Tailing Factor	Resolution
7.134	0.61	13981	1.06	13.46

*Mean values of six replicates

The calibration curve was prepared for the estimation of standard drug and its degradation product . The linearity (10.0-70.0μg/ml) was observed by linear regression equation method. The equation (AUC = mx + C) is AUC = 35648.62 X – 159.095 for piogilitazone HCl and correlation coefficient was found to be 0.999, indicating good linearity. After several trials it was observed that pioglitazone HCl got extensively degraded in 1N NaOH at the temperature of 80°C, and showed complete degradation in 30 minutes. In 0.1N NaOH , it degraded gradually, therefore degradation was observed in 0.1N NaOH at 80°C, at 226 nm by HPLC using mobile phase consisting of acetonitrile : 10mM potassium dihydrogen phosphate buffer (pH -6), (50:50v/v). The chromatogram of degraded sample showed well resolved peak of pioglitazone HCl and its various degradation products (Table 2) at different time intervals.

Table 2. Retention time of pioglitazone HCl and its degradation products

Compound	RT (min)
Pioglitazone HCl	7.0+0.2
Primary degradation product-I	2.4+0.1
Degradation Product –II	8.2+0.1
Diluent	1.4+0.1

Pioglitazone HCl was practically, completely degraded in 0.1 N NaOH at 80°C in 4 hours. Primary degradation product eluted at 2.4±0.1 minutes while pioglitazone HCl showed retention time of 7.0±0.2 minutes (Figure 2). As time proceeded, AUC for primary degradation product increased and for pioglitazone HCl it was decreased (Table 3). After 30 minutes of exposure to 0.1N NaOH at 80°C, 50 % of drug got degraded (Figures 3). In around 120 minutes primary degradation product further degraded and a secondary degradation product eluated at 8.2±0.1 minutes (Figures 4 and 5) . At 1.4±0.1 minutes, a peak corresponding to diluent was observed in all analysis.

Table 3. Comparative data of percentage of remaining Pioglitazone HCl and degradation product in 0.1 N NaOH at different time interval.

Time (min)	% of Degradation product	% of Remaining drug
0	0	100
5	01.18	98.82
10	04.20	95.81
15	10.01	89.99
20	15.88	84.12
30	29.68	70.31
40	40.44	59.56
50	50.04	49.96
60	56.95	43.05
75	67.69	32.31
90	74.15	25.85
120	84.36	15.64
150	88.63	11.37
180	91.78	08.21
210	95.21	04.79

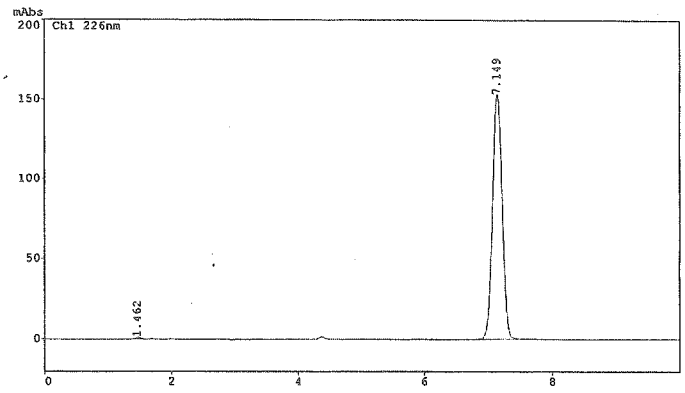

Figure 2. Chromatogram of 0 minute degradation of Pioglitazone HCl

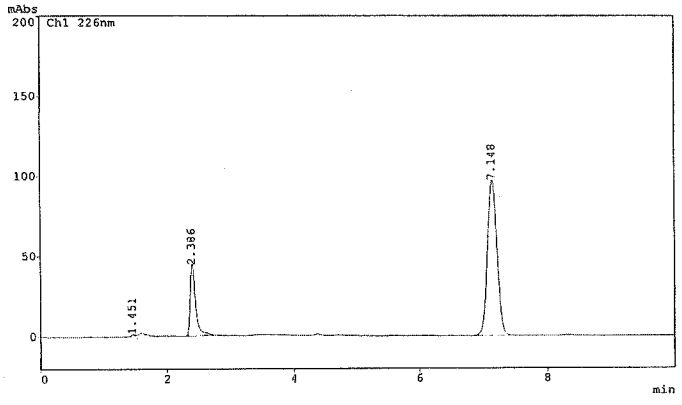

Figure 3. Chromatogram of 30 minute degradation of Pioglitazone HCl

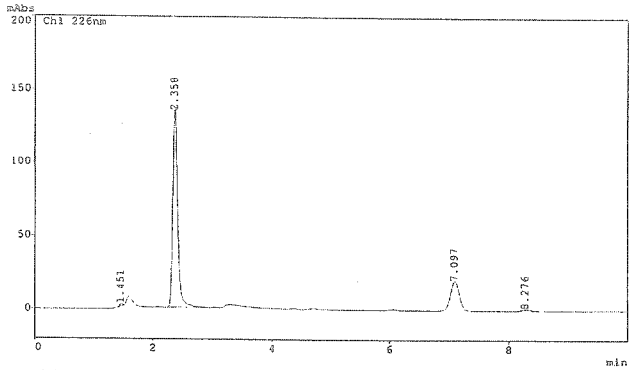

Figure 4. Chromatogram of 120 minute degradation of Pioglitazone HCl

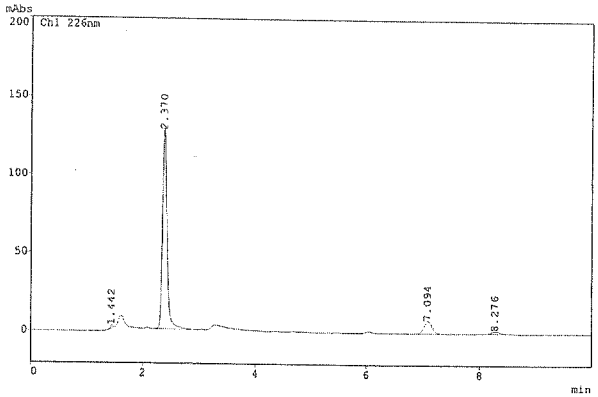

Figure 5. Chromatogram of 180 minute degradation of Pioglitazone HCl

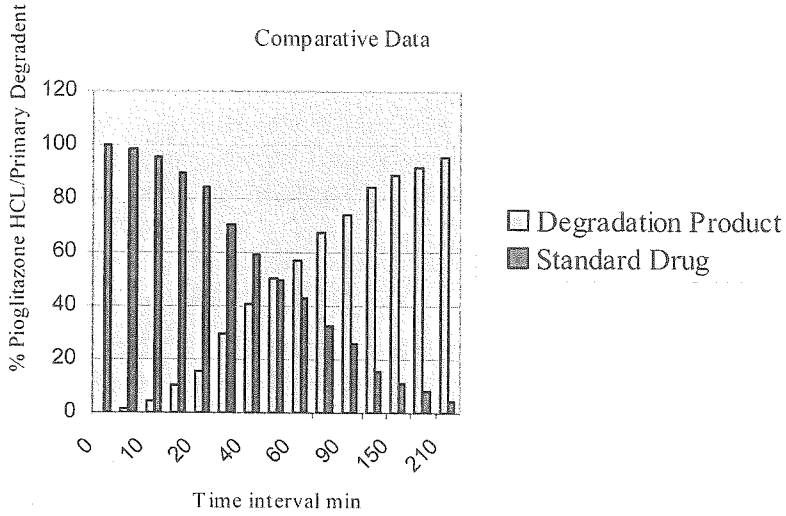

Figure 6. Graphical representation of percentage of remaining Pioglitazone HCl and degradation product in 0.1 N NaOH at different time intervals.

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