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Short Communication

Determination of Pioglitazone Hydrochloride in Tablets by High-Performance Liquid Chromatography

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Abstract

A rapid and accurate HPLC method has been developed for the determination of pioglitazone hydrochloride in tablets. Chromatographic analysis was performed on a Nova-Pak® C_{18} column (3.9mm x 150mm, 5µm) with a mixture of ammonium formate buffer adjusted with formic acid to pH 3 and acetonitrile (75:25, v/v) as mobile phase, at flow rate of 1.0 mL min^{-1}, and UV detection at 225 nm. The determination was completed in less than 12 min. Linearity ≥ 0.5 µgmL^{-1}, accuracy ≥ 99.14 %, and precision ≤ 0.6 % were found to be acceptable over the range 0.5 - 20 µgmL^{-1}.

Keywords: Pioglitazone, Tablets, HPLC.

Introduction

Pioglitazone hydrochloride (PG-HCl) (Fig.1), ((±)-5-{p-[2-(5-ethyl-2-pyridyl)ethoxy] benzyl}-2,4-thiazolidine-dione hydrochloride) is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus (also known as non-insulin-dependent diabetes mellitus [1] (NIDDM) or adult-onset diabetes). Pioglitazone decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Currently, it is marketed under the trad name Actos® [2]. An Actos® 30 mg tablet encapsulated in a gelatin capsule filled with lactose was developed for use as a placebo in clinical safety and efficacy studies.

Several HPLC methods have been reported for determining pioglitazone hydrochloride in tablets [3-6]. The quantitative determination of pioglitazone in human serum by direct-injection HPLC mass spectrometry and its application to a bioequivalence study has also been reported [7]. Yamashita determined pioglitazone and its metabolites in human serum and urine [8] and Zhang and Lakings reported an assay method for pioglitazone alone in dog plasma [9]. Potentiometric sensors [10] were fabricated for the determination of pioglitazone in some pharmaceutical formulations.

The aim of this study was to develop a rapid, economical, precise and accurate method for the determination of pioglitazone in tablets. The method described is quite suitable for the routine analysis of tablets.

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Experimental

Materials

Pioglitazone hydrochloride was received from Uni Pharma company, El Obour City, Cairo-Egypt. HPLC grade acetonitrile was obtained from SDS (de Valdonne, France). Analytical grade ammonium formate was purchased from Sigma-Aldrich, Germany. Formic acid was obtained from E. Merck, Darmstadt, Germany. Actos tablets were purchased from the market. Each Actos tablet consists of 30 mg of PG-HCl. Grade 1 water was obtained from a Mill-Q ultrapure water purification system (Millipore, Bedford, MA, USA).

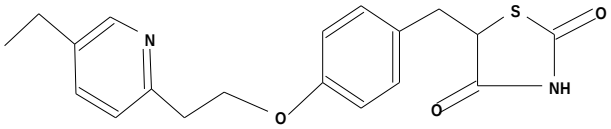

Figure 1. **Chemical Structure of Pioglitazone Hydrochloride. Molecular weight of Pioglitazone – free base is 356.5; elemental formula is $C_{19}H_{20}N_2O_3S$**

Instrumentation

High Performance LC

The LC system consisted of a Waters model 481 UV, a Shimadzu LC-6A pump, and injector

equipped (model 7125, Rheodyne, Berkeley California, USA) with 20 µL sample loop. The output signals were monitored and integrated using Perkin-Elmer Totalchrom software (version 6.2.1.).

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of aqueous 0.05M ammonium formate adjusted with formic acid to pH 3 and acetonitrile (75 : 25, v/v). Ammonium formate buffer of pH 3 (0.05 M) was prepared as follows [4] : 3.15 g of ammonium formate was dissolved in 950 ml water, pH was adjusted to value 3 ± 0.1 with formic acid (diluted with water in ratio 1:5), the buffer was diluted to 1000 mL with water and was filtered through a 0.45-µm (HVLP, Germany) membrane filter. The mobile phase was also filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. Symmetry C_{18} analytical column (3.9 mm x 150 mm, 5 µm packing), Waters state USA, was used for determination. The flow rate was 1.0 mL min^{-1} and the column was operated at ambient temperature (~25 ^{o}C). The volume of sample injected was 20 µL. The UV detector was set at a wavelength of 225 nm. A typical chromatogram of PG-HCl is shown in (Fig. 2).

Preparation of stock and working Standard Solutions

The stock solution of PG-HCl (500 µg mL^{-1}) was prepared by dissolving 0.05 g PG-HCl (99.78%) in methanol in 100-mL volumetric flask. Six different working/calibration solutions were prepared by taking different volumes from the stock solution and diluting with mobile phase. The concentration range of the working standard solutions was 0.5-20.0 µg mL^{-1}.

Sample Preparation

Ten tablets were weighed and their average weight was calculated. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent to one tablet (0.1754 g) which contains 30 mg of PG-HCl was weighed in a 100-mL volumetric flask, dissolved in methanol, and filtered through 0.45-µm (HVLP, Germany) filter paper. The filtrate (100, 233, 500 µL) was quantitatively transferred to three different 10-mL volumetric flasks, and the solutions were diluted to the volume with mobile phase.

Results and Discussion

Method development

Choice of stationary phase

To check column to column selectivity and ruggedness i.e. chromatographic variation due to the change of C_{18} column batch and manufacturer, the proposed chromatographic method is performed on two batches of C_{18} columns provided by Waters (3.9 mm x 150 mm, 5 µm) and Shandon, Hypersil (4.6mm x 150 mm, 5µm). Variations were observed in peak shape and retention time. C_{18} column provided by waters (3.9 mm x 150mm, 5µm) revealed better results. Bond broadening and greater noise was observed in the results obtained by C_{18} column provide band by Shandon, Hypersil (4.6 mm x 150 mm, 5µm).

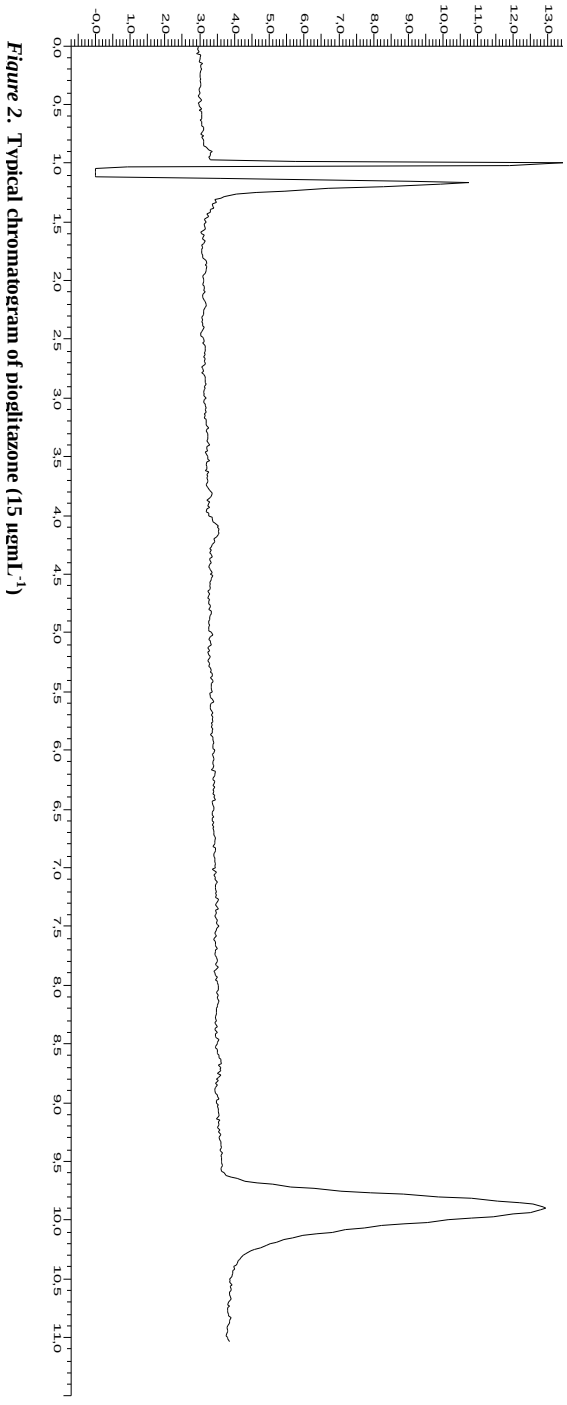

Figure 2. **Typical chromatogram of pioglitazone (15 µgmL^{-1})**

Choice of mobile phase

Good determination of PG-HCl was obtained when eluted with ammonium formate buffer of pH.3 and acetonitrile (75:25 v/v). PG-HCl was eluted after 10 min. However it was eluted after 3 min when the proportion of above mentioned mobile phase was changed to 55:45 v/v. So first proportion of mobile phase was preferred in order to achieve good analysis in case if there is any impurity in the sample. Second proportion of mobile phase may be used to shorten the analysis time.

Method Validation

Limits of Quantitation and Detection

The limit of quantitation (LOQ) was established at a signal-to-noise ratio of 10. The LOQ of PG-HCl was determined by six injections of the drug at the LOQ concentration. The LOQ of PG-HCl was found to be 0.5 µg mL^{-1}.

The limit of detection (LOD) was calculated according to [4].

$$LOD = \frac{3.3.\sigma}{S}$$

where σ is peak-to-peak noise of baseline in the chromatogram of blank solution, S is slope of the regression line acquired by measuring linearity. The limit of detection (LOD) of PG-HCl was 0.2 µgmL^{-1}. System suitability results of the method are presented in Table 1.

Table 1. **System-suitability results of HPLC method**

Compound	Retention time (min.)	Retention factor (k)	Selectivity (α)	Plates number (N)
Pioglitazone	10.1	5.73	3.3	6682

Linearity

Linearity was evaluated by analysis of working standard solutions of six different concentrations [11]. The millivoltage and concentration of the drug was subjected to regression analysis to calculate the calibration equation and correlation coefficients. The regression equation obtained was y = 0.6239x – 0.0652 (r = 0.9983, n = 6). The range of linearity was from 0.5 – 20.0 µg mL^{-1}. The results show that within the concentration range indicated above there was an excellent correlation between millivoltage and concentration of drug.

Accuracy

The accuracy of the method was determined by analyzing a sample obtained from mixing of ten tablets. Three solutions of concentration 3, 7 and 15 µgmL^{-1} were made in triplicate from this sample and analyzed for three consecutive days. Solutions for the calibration curves were prepared fresh every day. The assay accuracy variation shown in terms of relative mean error (RME), and % recovery are tabulated in Table 2 [11]. The RME values are below ±1.0 for the intra-day assay experiments. These results also show that the filtration of the samples can be performed without loss of analyte.

Table 2. **Accuracy in the assay of pioglitazone in tablets using HPLC.**

Day of analysis	Theoretical concentrations (µg mL^{-1})	Found Concentrations (µg mL^{-1}) (n=3)	% Recovery	% RME
1st day	3	2.9896	99.65	-0.35
	7	6.9401	99.14	-0.86
	15	15.011	100.07	0.07
2nd day	3	2.9965	99.88	-0.17
	7	6.9600	99.43	-0.57
	15	15.023	100.20	0.15
3rd days	3	3.0141	100.47	0.47
	7	7.0224	100.32	0.32
	15	15.1095	100.73	0.73

Precision

The precision of the method for the determination of PG-HCl was studied using the parameters i.e. repeatability, intermediate precision, and robustness. Repeatability in the intra-day variations in assay was obtained at different concentration levels. The precision is expressed in terms of RSD values calculated from the data of each day for three days. RSD values of assay were found to be below 0.5% (Table 3.). The intermediate precision, which is the inter-day variation at the same concentration level, was determined on successive days. The intermediate precision for assay of PG-HCl was found to be below 1.0% RSD, (Table 3.). The robustness of a method is a measure of its capacity to remain unaffected by small variations in method conditions. The robustness of the proposed method was evaluated by altering the pH of the mobile phase. The results indicated that there were no significant differences between the given conditions using the method developed, which is satisfactory for determination of PG-HCl in tablets.

Table 3. **Inter-and Intra-days assay variation of pioglitazone hydrochloride**

Day of analysis	Theoretical Concentrations (μgmL^{-1})	Actual Concentration (μgmL^{-1}) (n=3)	SD	% RSD
Intra-days:				
1st day	3	2.9896	0.005	0.156
	7	6.9401	0.027	0.386
	15	15.011	0.005	0.033
2nd day	3	2.9965	0.002	0.052
	7	6.9600	0.018	0.257
	15	15.023	0-010	0.068
3rd days	3	3.0141	0.006	0.209
	7	7.0224	0.010	0.143
	15	15.1095	0.049	0.324
Inter-day:	3	3.014	0.006	0.209
	7	6.961	0.017	0.250
	15	15.201	0.090	0.591

Stability of standard solutions

The stability of standard pioglitazone and sample solutions of tablet was determined by monitoring their peak-millivoltage over a period of two weeks [12]. The results showed that the retention time and peak-millivoltage were almost unchanged (RSD % <1.0) and no significant degradation occurred within the given period, indicating that solutions are stable for at least two weeks.

Method Application

The discussed HPLC method has been found valid for the determination of PG-HCl in tablets. The mean assay results, expressed as a percentage of the label claimed, are shown in Table (2 & 3).

Conclusion

This is an accurate, précised and selective HPLC method with short analysis time. It can be used for the determination of PG-HCl in tablets. Since interferences are not observed in this method, stronger mobile phase (55:45 v/v) may be used for shorter analysis time.

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References

1. A. Fahim, K.C. L. Nereida and M. R. Gerald, *Metabolism*, 58, 373 (2009) 378.
2. Physician's Desk Reference®, 54 ed., *Medical Economics Company*, Inc., Montivale, NJ, P. 3088 (2000) 3094.
3. R. T. Sane, S. N. Menon, S. Inamdar, M. Mote, and G. Gundi, *Chromatographia*, 59 (2004) 451.
4. A. Jedlička, J. Klimeš and Grafnetterova T., *Pharmazie*, 59, 178(2004)182.
5. B. L. Kolte, B. B. Raut, A. A. Deo, M. A. Bagool, and D. B. Shinde, *J.Chromatogr. Sci*., 42 (2004) 27.
6. Radhakrishna T., Sreenivas R.D., and Om R.G., *J. Pharma. Biomed. Anal*., 29 (2002) 593.
7. Y. J. Xue, K. C. Turner, J. B. Meeker, P. Janice, A. Mark, and U. Stere, *J. Chromatogr. B:*, 795 (2003) 215.
8. Y. Kenji, M. Hiromi, O. Teruaki and M. Michio, *J. Chromator. B:*, 677 (1996) 141.
9. S. Hidetaka, F. Yasuhiro and O. Terumasa, *J. Chromatogr.*, 670 (1995) 309 A.E.G.
10. Mostafa, A. Al-Majed, *J. Pharm. Biomed. Anal.*, 48, 57 (2008) 61.
11. ICH Validation of Analytical Procedures Methodology, *ICH Harmonised Tripartite Guidelines*, Adopted 6 NOV. (1996).
12. ICH Text on Validation of Analytical Procedures, *ICH Harmonised Tripartite Guidelines*, Adopted 27 OCT. (1994).