Asian Journal of Chemistry; Vol. 23, No. 10 (2011), 4500-4502

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

HPLC Estimation of Pioglitazone in Formulations and in Pharmacokinetic Studies

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(*Received*: 3 December 2010; *Accepted*: 24 June 2011) AJC-10085

A simple sensitive RP-HPLC method was developed and validated for the estimation of pioglitazone in bulk drug samples, its formulations and in plasma samples using RP C-18 column with UV detection at 269 nm. The mobile phase consists of a mixture of acetonitrile-water (60:40 % v/v) adjusted to pH 6.0 with 0.1 % v/v glacial acetic acid. The retention time for pioglitazone was 5.4 min. The intra-and inter-day coefficient of variation was less than 1.28 % showing high precision of the method. The method was highly accurate with a recovery in the range 99-100 %. The method was found suitable for estimating pioglitazone in plasma samples in pharmacokinetic studies.

Key Words: HPLC method, Pioglitazone, Pharmacokinetics.

INTRODUCTION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention. Pioglitazone, (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy] benzyl)thiazolidine-2,4-dione, is an oral hypoglycemic drug, belonging to thiazolidine diones class, which is used in type II diabetes. So far very few liquid chromatography (LC) procedures have been reported for the determination of pioglitazone in biological fluids[1-3]. Different analytical methods including, capillary electophloresis[4-7], TLC[8], HPLC[9], ultraviolet detection[10] and mass spectroscopy[11] have been reported for determination of pioglitazone in pharmaceutical dosage forms and in biological fluids. The objective of the present study is to develop a simple, rapid and sensitive HPLC method for the analysis of pioglitazone in bulk drug samples and its formulations using most commonly employed RP-C_{18} column with UV detection, which would serve as reliable and rapid method for the determination of pioglitazone in bulk drug and in pharmaceutical formulations.

EXPERIMENTAL

Pioglitazone was a gift sample from M/s. Orchid Health Care, Chennai. Methanol HPLC grade (Merck), acetonitrile HPLC grade (Qualigens Fine Chemicals), glacial acetic acid HPLC grade (Qualigens Fine Chemicals) and water HPLC grade (Qualigens Fine Chemicals) were procured from commercial sources. All other materials used were of pharmacopoeial grade.

Chromatographic conditions: The HPLC system (make: M/s Shimadzu Corporation, Japan.) consisted of UV-Visible detector (Shimadzu, model: SPD-10 AVP), C-18 column (Phenomenex, DESC: Gemini 5µ C18 110A, Size: 250 × 4.6 mm, S/No: 288063-23), 2 pumps (Model: LC-10 ATVP) and a micro syringe of capacity 25 µL (Model: Microliter® # 702, Mfd. by: M/s Hamilton) were used.

Mobile phase: The mobile phase consists of a mixture of acetonitrile-water (60:40 % v/v) adjusted to pH 6.0 with 0.1 % v/v glacial acetic acid. The mobile phase was filtered through 0.45 µ membrane filter before use and was run at a flow rate of 1 mL/min.

Preparation of standard solutions: Pioglitazone (50 mg) was dissolved in 50 mL of acetonitrile in a volumetric flask. The above solution was further diluted with diluent (acetonitrile: water) to get a concentration of 20 µg/mL (stock solution). The stock solution of pioglitazone was further diluted with the mobile phase to get various concentrations namely 1, 2, 4, 6, 8 and 10 µg/mL. Each drug solution was filtered through 0.45 µ memberane filter before use and 20 µL of each were injected into the column.

Detection: The column effluent was monitored at 269 nm.

Accuracy: Accuracy of HPLC assay method was assessed by adding known amount (20 µg) of drug to drug solution of known concentration (10 µg/mL) and subjecting the samples to the proposed HPLC method. The study was replicated 4 times. The accuracy was expressed in terms of the recovery and calculated by multiplying the ratio of measured drug concentration to the theoretical concentration with 100, so as to give per cent recovery.

Precision: The precision of the HPLC method was determined in terms of intra-and inter-day variation in the peak

areas for a set of drug solutions (2 or 4 µg/mL) assayed 4 times on the same day and on 3 different days. The intra-and inter-day variations in the peak areas of drug solutions was calculated in terms of coefficient of variation.

Estimation of pioglitazone in formulations: For the estimation of pioglitazone in commercial tablet formulations, 10 tablets in each case were weighed and finely powdered. Accurately weighed tablet powder equivalent to 50 mg of pioglitazone was taken into a 25 mL volumetric flask containing 20 mL acetonitrile and the solution was filtered through 0.45 µ membrane filter. The solution was suitably diluted with mobile phase to produce a concentration of 4 µg/mL and 20 µL of each were injected into the column for assay.

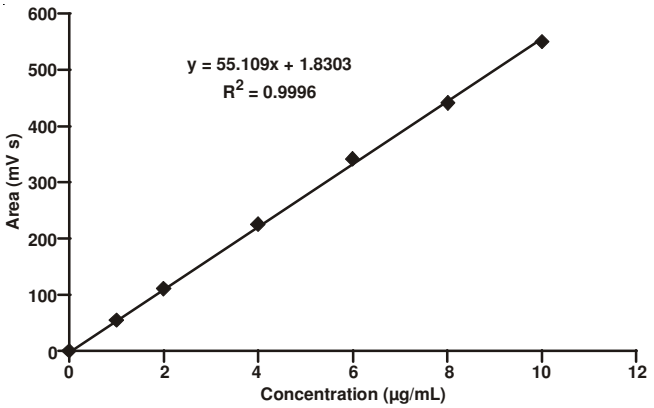

Fig. 1. Calibration curve for the estimation of pioglitazone by the HPLC method developed

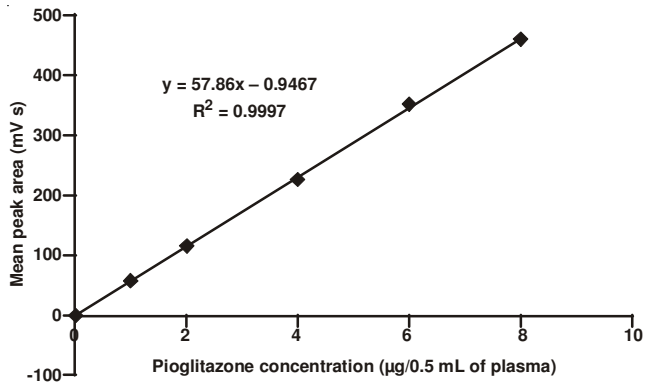

Fig. 2. Calibration curve for the estimation of pioglitazone in plasma samples by the HPLC method development

Estimation of pioglitazone in plasma: The HPLC method developed was used for the estimation of pioglitazone in plasma samples. For this purpose a calibration curve was constructed by analyzing plasma samples (Fig. 2) containing different amounts of pioglitazone as follows.

To a series of tubes containing 0.5 mL of plasma in each, 0.1 mL drug solution containing 1, 2, 4, 6 and 8 µg of pioglitazone were added and mixed. To each tube 1 mL of acetonitrile was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5 mL) was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5 mL of mobile phase was added and mixed for reconstitution. Subsequently 20 µL were injected into the column for HPLC analysis.

Pharmacokinetic study: The *in vivo* protocols were approved by Institutional Animal Ethics Committee (Regd. No. 516/01/a/CPCSEA). Healthy rabbits of either sex (n = 6) weighing 1.5-2.5 Kg were fasted over night. After collecting the zero hour blood sample (blank), pioglitazone was administered at a dose of 5 mg/kg of body weight orally with 10 mL of water. Blood samples (3 mL) were collected from marginal ear vein at 1,2,4,6,8,12,16,20 and 24h after administration. Samples were collected in heparinized tubes and were centrifuged at 10000 rpm for 10 min. The plasma separated was collected into dry tubes and the samples were stored under refrigerated conditions prior to assay for pioglitazone on the same day. Plasma concentrations of pioglitazone were determined by the HPLC method developed as described above. From the time *vs* plasma concentration data (Fig. 3) various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant, (K_{el}), biological half life ($t_{1/2}$), per cent absorbed to various times and absorption rate constant (K_a) were calculated in each case as per known standard methods[12].

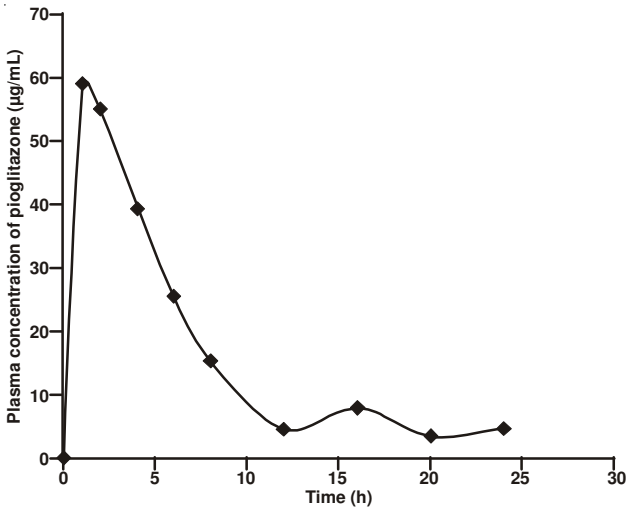

Fig. 3. Time *vs* plasma concentration profile following the oral administration of pioglitazone in rabbits

RESULTS AND DISCUSSION

The mobile phase consists of a mixture of acetonitrile-water (60:40 % v/v) adjusted to pH 6.0 with 0.1 % v/v glacial acetic acid. The retention time for pioglitazone was 5.4 min. The peak areas at different concentrations were reproducible as indicated by low coefficient of variation (1.25 %). When the concentrations of pioglitazone and their peak areas were subjected to regression analysis by least squares method, a good linear relationship (r = 0.9996) was observed between the two in the range 1.0-20 µg/mL. The regression of pioglitazone concentration (µg/mL) over its peak area was found to be y = 1.8303 + 55.109x, where y is peak area and x is concentration of pioglitazone (µg/mL). This regression was used to estimate the amount of pioglitazone in formulations.

The HPLC method developed was validated for intra- and inter- day variations. When the solutions containing 2 and 4 µg/mL of pioglitazone were injected repeatedly on the same day and on other days, the coefficient of variation in amounts

estimated was less than 1.28 % (Table-1). The results indicate that the HPLC method is highly reproducible. In the accuracy assessment, the recovery was found to be 99-101 % (Table-2). Thus the method is highly accurate.

TABLE-1
PRECISION OF THE PROPOSED HPLC METHOD

Pioglitazone concentration (μg/mL)	Amount of pioglitazone (μg/mL) found on	
	Inter-day (% CV) (n = 4)	Intra-day (% CV) (n = 4)
2	1.996 (0.029)	1.994 (0.28)
4	3.971 (1.280)	3.960 (1.06)

TABLE-2
RECOVERY OF PIOGLITAZONE

Amount of drug added (μg)	Mean (± SD) Amount (μg) recovered (n = 4)	Mean (± SD) % of recovery (n = 4)
20	19.94 ± 0.53	99.74 ± 1.59

TABLE-3
ASSAY OF PIOGLITAZONE COMMERCIAL BRANDS

Brand name	Labeled amount (mg)	Estimated amount (mg ± sd)	Drug content (%)
Piotaz	30	29.35 (1.03)	97.83
Piosafe	30	29.48 (1.11)	98.26

The HPLC method developed has also been used for assay of two commercial brands of pioglitazone. The drug content of the commercial tablets was found to be 97.83-98.26 % of the labeled amount (Table-3). No interfering peaks were found in the chromatograms of tablet assay indicating that excipients used in the tablet formulations tested did not interfere with the estimation of drug by the HPLC method developed.

The HPLC method developed was also tested for its application in pharmacokinetic studies. Plasma samples containing known amounts of pioglitazone were analyzed by the HPLC method developed. A good linear relationship was observed between plasma concentration of pioglitazone and peak area. The relationship could be expressed by the following linear equation:

$$y = 57.86 \times - 0.9467$$

where x is plasma concentration of pioglitazone (μg/0.5 mL of plasma) and y is peak area. This linear regression equation was used for estimating plasma concentrations of pioglitazone in the *in vivo* pharmacokinetic study. The precision (RSD) of the method less than 0.48 %.

The pharmacokinetic parameters estimated following the oral administration of pioglitazone are given in Table-4. The elimination rate constant (K_{el}) for pioglitazone was found to be 0.1126 h^{-1} and the corresponding half life was found to be 6.15 h following the oral administration of pioglitazone. The $t_{1/2}$ value of pioglitazone obtained in the present work is in good agreement with the earlier reported[13] value of 3-7 h. The mean residence time (MRT) was found to be 8.99 h. The absorption rate constant (K_a) was found to be 0.3539 h^{-1}. A C_{max} of 4.10 ± 0.12 μg/mL was observed at 2.0 h after oral administration of pioglitazone. Later the plasma concentrations were decreased rapidly. The pharmacokinetic parameters of pioglitazone estimated employing the HPLC method developed agreed well with the literature values.

TABLE-4
PHARMACOKINETIC PARAMETERS ESTIMATED FOLLOWING THE ORAL ADMINISTRATION OF PIOGLITAZONE

Pharmacokinetic Parameter	Estimated value
C_{max} (μg/mL)	4.10 ± 0.12
T_{max} (h)	2.0
K_{el} (h^{-1})	0.1126
$t_{1/2}$ (h)	6.15
$(AUC)_0^{24}$ (μg h/mL)	48.58
$(AUC)_0^{\infty}$ (μg h/mL)	53.55
K_a (h^{-1})	0.3539
MRT (h)	8.99

Conclusion

A simple, accurate and sensitive RP-HPLC method was developed and validated for the estimation of pioglitazone in bulk drug samples, its formulations and in plasma samples using RP C-18 column with UV detection at 269 nm. The intra and inter day coefficient of variation was less than 1.28 % showing high precision of the method. The method was highly accurate with a recovery in the range 99-100 %. The method was found suitable for estimating pioglitazone in formulations and also plasma samples in pharmacokinetic studies.

REFERENCES

1 W.Z. Zhong and D.B. Laking, *J. Chromatogr. Biomed. Appl.,* **490**, 377 (1989).
2. K. Yamashita, H. Murakami, T. Okuda and M. Motohashi, *J. Chromatogr. B*, **677**, 141 (1996).
3. W.Z. Zhong and M.G. Williams, *J. Pharm. Biomed. Anal.*, **14**, 465 (1996).
4. S. Hillaert and W. Van den Bossche, *J. Pharm. Biomed. Anal.* **25**, 775 (2001).
5. E.C. Gli, A.V. Schepdael, E. Roets and J. Hoogmartens, *J. Chromatogr. A*, **895**, 43(2000).
6. S.-W. Sun and A.-C. Wu, *J. Liq. Chromatogr. Relat. Technol.,* **22**, 281 (1999).
7. J.J. Berzas Nevado, G.C. Panalvo and F.J. Guzman Bernardo, *J. Liq. Chromatogr. Relat. Technol.*, **22**, 1975 (1999).
8. A Gumieniczek, H. Hopkala and A. Berecka, *J. Liq. Chromatogr. Relat. Technol.*, **27**, 2057 (2004).
9. P.K. Chaturvedi and R. Sharma, *Acta Chromatogr.,* **203**, 451(2008).
10. P. Sripalakit, P. Neamhom and A. Saraphanchotiwitthaya, *J. Chromatogr. B*, **843**, 164 (2006).
11. Y.J. Xue, K.C. Turner, J.B. Meeker, J. Pursley, M. Arnold and S. Unger, *J. Chromatogr. B*, **795**, 215 (2003).
12. J.G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53**, 1392 (1964).
13. Product Leaflet, ACTOS™ (Pioglitazone Hydrochloride) Tablets, NDA No. 21-073.