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HPLC Method for the Determination of Pioglitazone in Human Plasma and Its Application to Pharmacokinetic Study

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For the quantification of pioglitazone in human plasma a validated HPLC method was developed using ultraviolet detection. Chromatographic separation of pioglitazone in plasma was achieved on a C_{18} column. Mixture of acetonitrile and (0.1 M) ammonium acetate (41:59) having pH (4.10) was used as a mobile phase. Piroxicam was used as an internal standard. The calibration curve was linear over the range 0.055-2.0 μg/mL in human plasma (r^2 = 0.9986). After oral administration of pioglitazone (30 mg) to male volunteers, the plasma concentration-time curve of pioglitazone was best conformed to two-compartment open model. The maximum concentration (C_{max}) 0.86 μg/mL was achieved at the peak concentration time (t_{max}) 2.40 h. AUC_{0-24} of pioglitazone was observed 7.71 h μg/mL. Total body clearance, volume of distribution and elimination rate constant of pioglitazone after oral administration were 4.84 L/h, 52.48 L and 0.45 L/h, respectively.

Key Words: Pioglitazone, HPLC, Volunteer, Validation, Pharmacokinetics.

INTRODUCTION

Pioglitazone (±) 5-{[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl}-2,4-thiazolidinedione is an oral anti-hyperglycemic agent which is used in the treatment of type-II diabetes mellitus[1]. It is from the class thiazolidinediones represent a potentially important group of drugs with a mechanism of action differing from the existing therapies[2]. It acts primarily by decreasing insulin resistance in the treatment of type-II diabetes[3]. It is a peroxisome proliferator activated receptor (PPARγ) agonist that increases transcription of insulin responsive genes and thus increases insulin sensitivity[4,5].

Previous clinical trials in healthy subjects have shown that pioglitazone is well absorbed after oral administration. The peak concentrations of pioglitazone in the blood of healthy subjects are achieved *ca.* 1.5 h after oral drug administration. It is highly bound to plasma proteins (*ca.* 97 %), with a low tissue distribution and slow elimination (half-life *ca.* 9 h). It is extensively metabolized in the liver, with the majority excreted as inactive metabolites in the faeces[6]. Pioglitazone is partially metabolized to various metabolites to exert its pharmacological activity[7,8].

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The environmental conditions under various geographical locations influence the genetic characters of the population living in that area. These geonetical influences are characterized by physiological and biochemical manifestation which are peculiar to the population. These differences have significant influences on bio-disposition, pharmacokinetics and elimination of various drugs and ultimately affect the response to the drugs[9].

There is limited literature on the pharmacokinetics/biokinetics of pioglitazone in Pakistani volunteers. Therefore the present project was design to develop a sensitive and precise analytical method for determining pioglitazone and to evaluate pharmacokinetic parameters in human beings. This study will help us to adjust the quantity and frequency of dose under local conditions.

EXPERIMENTAL

The concentration of pioglitazone in blood was determined by high performance liquid chromatography (HPLC) as described by Zhang *et al.*[10] with some modifications. The modification included the use of internal standard, change in pH (for good peak resolution) and different mobile phase ratio (for change in the retention time).

Pioglitazone and piroxicam standard were gifted by Hilton Pharma (Pvt.) Ltd., Karachi, Pakistan. Ethyl acetate, acetic acid and dimethyl sulphoxide (DMSO) were used by Merck (Darmstadt, Germany). HPLC-grade acetonitrile and methanol were used by Panreac (Barcelona, Spain). Dipotassium hydrogen phosphate (K_2HPO_4) was used by Fisher Scientific (Loughborough, Leicestershire, UK). Blank plasma was collected from Chiniot Dialysis Center, Faisalabad, Pakistan.

In this study the drug pioglitazone commercially known Actos®, manufactured by (The Arab Pharmaceutical Manufacturing Co. Ltd., Sult-Jordan, under license of Takeda Chemical Industries Ltd., Osaka, Japan, Batch No. 856029) was used in the dosage form of oral tablets 30 mg each.

Analytical procedure

The standard stock solution (100 µg/mL) of each pioglitazone and piroxicam were prepared by dissolving them in dimethyl sulphoxide and methanol, respectively. Working standard solutions were prepared by the appropriate dilutions of the above mentioned standard stock solution. A series of standard solutions containing both the drug and the internal standard were also prepared. The standard stock solutions were stored in the dark under refrigeration.

Shimadzu high performance liquid chromatography (HPLC) system (LC-10A) equipped with a fixed wavelength UV-Vis detector (Model SPD 10A, Shimadzu Corporation, Kyoto, Japan), column oven CTO 10A, liquid pump (LC-10AS) and acquisition software (Class LC-10) was used for the qualitative and quantitative determination of pioglitazone. The analytical column used to achieve chromatographic separation was a stainless steel (C_{18}) column, Discovery Supelco, Cat. No. 568523 (25 cm × 4.6 mm, 5 µm) Bellefonte (USA) protected by a guard column of the same material.

All the chromatographic analysis was carried out at 30 °C. The compounds were separated isocratically with a mobile phase consisting of acetonitrile and (0.1 M) ammonium acetate (41:59). Before use, the mobile phase was filtered by passing through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and was sonicated through sonicator (Cole-Palmer, USA) for 10 min. A constant flow rate of 1.0 mL/min was maintained. The effluent was monitored spectrophotometrically at 269 nm.

Sample preparation: In a 10 mL glass tube, 1.0 mL plasma sample, 50 μL of internal standard (20 μg/mL) and 250 μL 0.1M dipotassium hydrogen phosphate (K_2HPO_4) was vortexed on a vortex mixer (Scientific Industries, Inc., NY, USA) for 30 s. Then 5 mL ethyl acetate was added into the tube. This mixture was again vortexed for 3 min and centrifuged at 2130 x g (Shanghai Allcan Medical Co. Ltd, China) for 5 min. After centrifugation, 4 mL of organic layer was removed into another tube and was evaporated to dryness under a nitrogen stream at 45 °C in a water bath. The residue was reconstituted in 150 μL mobile phase. From this 20 μL was directly injected onto the HPLC column.

Choice of internal standard: In the present analysis piroxicam was used as internal standard because it was stable and did not interfere with the matrix of pharmaceutical samples. Moreover, it was well separated from pioglitazone. A sharp and symmetrical peak was obtained with good baseline for each compound, thus facilitating the accurate measurement of the peak area. Under the described HPLC parameters, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times.

The chromatogram showed no interference from the endogenous substances. HPLC chromatograms of pharmaceutical formulation, blank plasma, plasma spiked with internal standard and pioglitazone have been shown in Fig. 1. The retention times of pioglitazone and the internal standard in plasma were 7.91 and 10.22 min, respectively.

Method validation: The method was validated according to Good Laboratory Practice standards to achieve the reliable results that could be properly interpreted. The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in guidelines[11,12]. The method was validated for linearity, accuracy, precision/repeatability (intra-day and inter-day), specificity and stability. Furthermore, this method is rapid, reliable and economical.

Pharmacokinetics: The plasma concentration of the drug from each volunteer was plotted on a normal logarithmic scale against time. On the basis of plasma concentration *versus* time data the pharmacokinetic parameters were determined using the PC-Computer Program, APO, MWPHARM version 3.02, a MEDIWARE product, Holland.

The plasma concentration of pioglitazone was measured by HPLC method in the plasma samples from the blood collected at time intervals 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10, 12, 24 and 36 h after oral administration of 30 mg tablet in 24 healthy male volunteers. In all the samples collected at 36 h no drug was quantified. Therefore, the data has been calculated on the basis of last detectable concentration time.

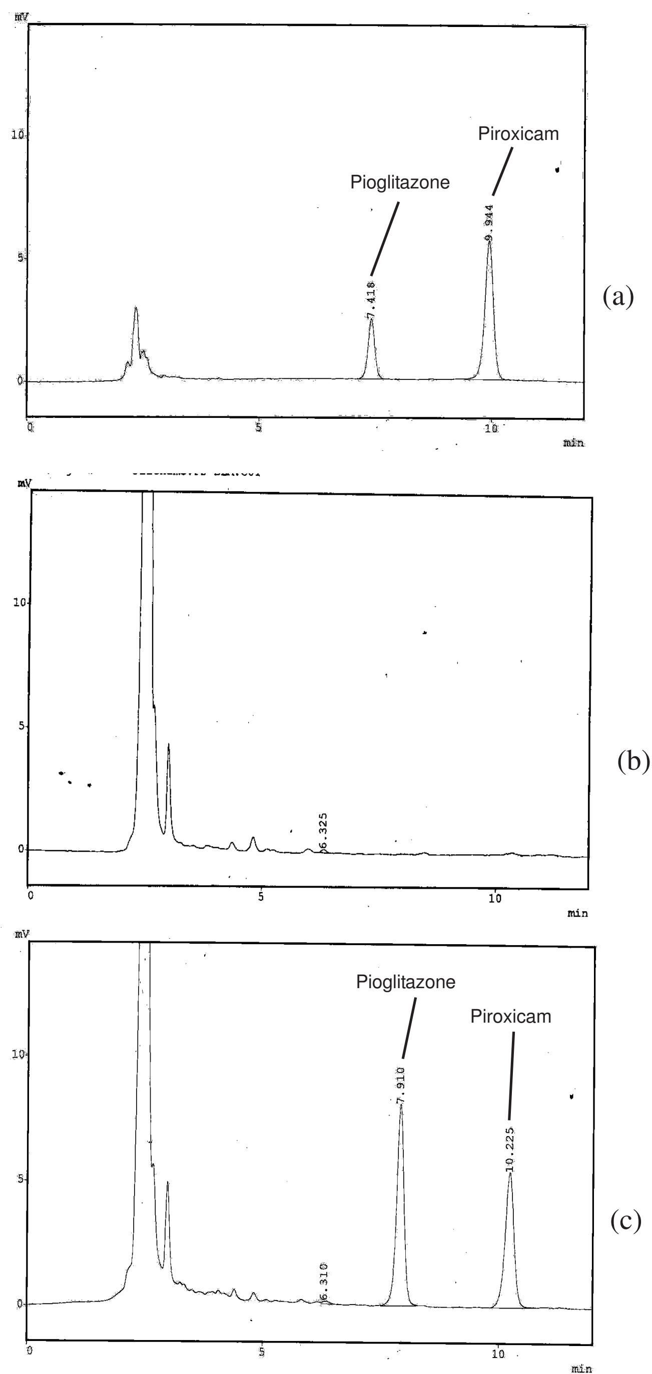

Fig. 1. Representative HPLC chromatograms of pioglitazone and internal standard in pharmaceutical formulation (a) blank plasma (b) plasma spiked with pioglitazone and internal standard (c)

Statistical methods: For computation and analysis of the drug plasma concentrations *versus* time data and the graphics, the computer software Microsoft Excel 7.0 was used. All data are reported as the mean ± SE. as described by Steel *et al.*[13].

RESULTS AND DISCUSSION

Validation of analytical method

Limits of detection and quantification: The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal-to-noise ratio. For LOQ a signal at least more largely than five times of the base line noise detected with a sufficient precision (< 20 %) and accuracy (80-120 %). The LOD obtained for plasma samples was 25 ng/mL and LOQ was 55 ng/mL.

Selectivity: Selectivity of the analyte was determined by spiking the drug in blank plasma in graded concentration and ascertaining from the retention time.

Specificity: Under the method and chromatographic conditions used, no interferences were noted in the control samples around the retention time of pioglitazone and piroxicam.

Linearity: Working standard solutions of pioglitazone were used for the evaluation of linearity. The linearity was investigated by linear regression analysis, which was calculated by the least square regression method. The calibration curve was constructed by plotting concentration *versus* area ratio. The linearity of the method was determined over a concentration range 0.055-2.0 μg/mL in plasma samples. The calibration curve of pioglitazone in human plasma was described by the following equation: $y = 0.9345x + 0.015$, $r^2 = 0.9986$

Recovery: Recovery was determined by comparing concentration from extracted quality controls with those obtained after injection of known volumes of stock solution prepared and processed in a similar manner as the quality controls. The recovery was examined for low, medium and high concentration ranges in plasma samples and was quite similar and consistent, independent of the concentration range. The average recovery of the assay was more than 79 %.

Precision/reproducibility and accuracy: The accuracy was expressed as the percentage of analytes recovered by the assay. System precision was determined from results of six replicate injections of the quality control samples. The precision (intra-day and inter-day) and accuracy of the assay was ascertained based on analysis of quality control samples. Quality control samples of plasma at each concentration were analyzed on three consecutive days, after which intra- and inter-day means, standard deviations and coefficient of variation (CV % or RSD) were calculated by standard methods[12]. The intra-day and inter-day precision in plasma are depicted in Table-1.

Stability: No significant degradation of pioglitazone under the studied concentrations was observed in plasma. The prepared sample extracts were stable for at least 24 h at room temperature. The long-term stability was evaluated after keeping the plasma samples frozen at -20 ºC for 2 months. The stability of standard and

TABLE-1
INTRA- AND INTER-ASSAY PRECISION AND ACCURACY
FOR PIOGLITAZONE IN PLASMA

Reproducibility	Concentration (µg/mL)		CV (%)	Recovery (%)
	Added	Found (mean ± SD)		
Intra-assay reproducibility[a]				
	0.10	0.08 ± 0.01	1.99	80.59
Quality controls	0.75	0.59 ± 0.01	1.78	80.12
	1.50	1.23 ± 0.02	2.27	79.50
Inter-assay reproducibility[b]				
	0.10	0.08 ± 0.01	2.45	78.34
Quality controls	0.75	0.60 ± 0.01	1.79	80.54
	1.50	1.21 ± 0.01	1.53	79.78

[a]Six quality control samples per concentration.
[b]Eighteen quality control samples per day (six per concentration) for 3 days.

sample solutions was determined by monitoring the peak-area ratio of solutions of standard pioglitazone over a period of three months at 4 ºC. The retention times and peak area ratios of pioglitazone was almost unchanged and that no significant degradation is observed within the given period. These studies suggested that human plasma samples containing pioglitazone can be handled under normal laboratory conditions without significant loss of compound.

Pharmacokinetic/biokinetic parameters: The pharmacokinetic parameters of pioglitazone after oral dose of 30 mg tablet were determined in 24 healthy male volunteers. Plasma concentrations of pioglitazone *versus* time profile of each volunteer were used to determine the pharmacokinetics. Average pharmacokinetic parameters of each volunteer are presented in Table-2. Following oral administration of 30 mg pioglitazone to 24 male volunteers the maximum concentration (C_{max}) 0.86 ± 0.06 µg/mL was achieved at the peak concentration time (t_{max}) of 2.40 ± 0.18 h. Average area under the plasma concentration-time curve (AUC) of pioglitazone was 7.71 ± 0.67 h µg/mL. Total body clearance, volume of distribution and elimination rate constant of pioglitazone after oral administration were 4.84 ± 0.62 L/h, 52.48 ± 10.63 L and 0.45 ± 0.09 L/h, respectively. Average absorption and elimination half-life values were 1.24 ± 0.20 h and 8.83 ± 2.01 h respectively.

The LOD and LOQ, good linearity and reproducibility of this method for different parameters indicate that it is the rapid and reliable method for the determination of pioglitazone in human fluids. Further the statistical evaluation of the proposed method led us to the excellency of this method. Different columns were checked: Capcell Pak C_{18} SG120 (150 × 4.6 mm i.d.) Shiseido, Tokyo, Japan), Luna C_{18} (5 µm, 25 cm × 4.6 mm i.d.) phenomenex, USA, Discovery Supelco C_{18}, Cat. No. 568523 (25 cm × 4.6 mm, 5 µm) Bellefonte (USA). Of these columns, only Discovery Supelco C_{18}, Cat. No. 568523 (25 cm × 4.6 mm, 5 µm) Bellefonte (USA) gave a sharp peak with a minimum tailing. This method is rapid and simple as compared to the methods described in literature[14,15].

TABLE-2
PHARMACOKINETIC PARAMETERS OF PIOGLITAZONE AFTER ORAL DOSE OF 30 mg TABLET TO HUMAN MALE VOLUNTEERS

Parameters	Mean	± SD
Area Under the Curve (AUC) [h µg/mL]	7.71	0.67
AUC polyexponential (t = 24)	6.56	0.59
AUC trapezoidal rule (t = 24)	6.51	0.60
Clearance (CL) [L/h]	4.84	0.62
Volume of distribution comp. 1 [L]	19.89	3.36
Volume of distr. steady state [L]	35.76	5.53
Volume of distribution [L]	52.48	10.63
Half-life phase 1 [h]	1.14	0.13
Half-life phase 2 [h]	8.83	2.01
Rate constant k10 [L/h]	0.29	0.03
Rate constant k12 [L/h]	0.27	0.07
Rate constant k21 [L/h]	0.45	0.09
Mean Residence Time (MRT) [h]	10.72	1.45
Absorption rate constant (ka) [L/h]	1.27	0.38
Absorption half-life [h]	1.24	0.20
Lag-time [h]	0.42	0.06
Time to peak T_{max} [h]	2.40	0.18
Peak concentration C_{max} [µg/mL]	0.86	0.06

In this method, pH of the mobile phase is an important factor. A very small change in pH disturbed the retention time to a very large extent. Therefore pH was kept at 4.10 ± 0.01 and was adjusted with the help of acetic acid (1 M). The plasma concentration of pioglitazone showed a rapid absorption until 2.0 h and then declined gradually and could not be detected in the plasma samples collected at 36 h (Fig. 2).

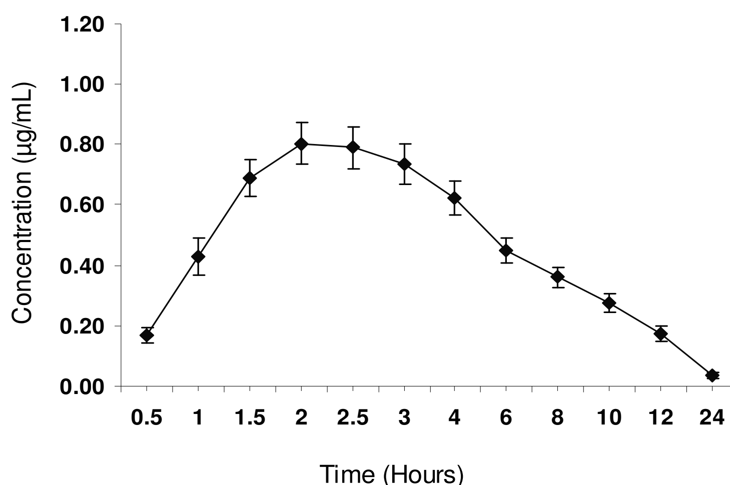

Fig. 2. Plasma concentration mean ± SE (µg/mL) of pioglitazone after oral dose of 30 mg tablet to 24 healthy male volunteers

After oral administration of 30 mg pioglitazone tablet in male volunteers the maximum concentration (C_{max}) 0.86 µg/mL was achieved at the peak concentration time (t_{max}) of 2.40 h. This value of C_{max} is less as compared to the value (1.329 µg/mL) calculated by Budde *et al.*[16] in male after oral administration of 1.5 time higher (45 mg) dose but with half dose (15 mg) the value of C_{max} was 0.524 µg/mL[17] which is less than the value described in the present study.

There are also differences and similarities in the values of t_{max} calculated in the present study and values present in the literature. The value is comparable with the values 2.0 and 2.3 h calculated by earlier researchers[16,17]. Sripalakit *et al.*[14] investigated t_{max} value 1.5 h for pioglitazone which is less as compared to present findings.

In present study average area under curve (AUC) of pioglitazone was observed 7.71 h µg/mL. This value was less as compared to calculated values 10.2 h µg/mL[18], 11.86 h µg/mL[14] and greater than 4.59 µg h/mL[17]. The elimination half-life of pioglitazone was 8.83 h. This value is greater than the values 5.2 h[14] and 6.5 h[18].

The values of volume of distribution and total body clearance calculated in present study were 52.48 L (0.89 L/Kg) and 4.84 L/h, respectively which were higher than the values 0.253 L/kg and 2.4 L/h reported in the literature[6].

All this indicates that both differences and similarities are present in the values of different calculated parameters and the values present in the literature. These similarities and minor differences may be attributed to various genetical and environmental factors. Along with further studies these calculated parameters will support to adjust the quantity of dose in the indigenous population.

Conclusion

A simple, specific and sensitive HPLC method for the determination of pioglitazone in human plasma and pharmaceutical formulation was developed, which was applied to pharmacokinetic investigation in human after oral administration of 30 mg pioglitazone. Application of the method to pharmacokinetic studies has been successful. Similarities and minor differences are present in the values of the different biokinetic parameters calculated in our study and the values reported in the literature.

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REFERENCES

1. P.S. Gillies and C.J. Dunn, *Drugs*, **60**, 333 (2000).
2. H. Yki-Jarvinen, *N. Engl. J. Med.*, **351**, 1106 (2004).
3. T. Radhakrishna, D.R. Sreenivas and G.O. Reddy, *J. Pharm. Biomed. Anal.*, **29**, 593 (2002).
4. M. Fuchtenbusch, E. Standl and H. Schatz, *Exp. Clin. Endocrinol. Diabetes*, **108**, 151 (2000).

5. G.H. Scholz, W.A. Scherbaum and G. Lubben, *Diabetes Stoffwechsel.*, **9**, 23 (2001).
6. D.A. Eckland and M. Danhof, *Diabetes*, **108**, 234 (2000).
7. Y. Kiyota, T. Kondo, Y. Maeshiba, A. Hashimoto, K. Yamashita, Y. Yoshimura, M. Motohashi and S. Tanayama, *Arzneimittelforschung*, **47**, 22 (1997).
8. Y. Maeshiba, Y. Kiyota, K. Yamashita, Y. Yoshimura, M. Motohashi and S. Tanayama, *Arzneimittelforschung*, **47**, 29 (1997).
9. M. Nawaz, T. Iqbal and R. Nawaz, *Vet. Pharmacol. Toxicol. Therapy Food Prod. Anim.*, **2**, 260 (1988).
10. H. Zhang, X. Wang, X. Zhang, Q. Zhang, Q. Yin and K. Li, *Asian J. Drug Metabol. Pharmacokinetics*, **4**, 119 (2004).
11. ICH Harmonized Tripartite Guidelines (1996).
12. FDA Guidance for Industry (1998).
13. R.G.D. Steel, J.H. Torrie and D.A. Dickey, McGraw Hill Book Co., Inc., New York (1997).
14. P. Sripalakit, P. Neamhom and A. Saraphanchotiwitthaya, *J. Chromatogr. B*, **843**, 164 (2006).
15. R.T. Sane, S.N. Menon, S. Inamdar, M. Mote and G. Gundi, *Chromatographia*, **59**, 451 (2004).
16. K. Budde, H.H. Neumayer, L. Fritsche, W. Sulowicz, T. Stompor and D. Eckland, *Br. J. Clin. Pharmacol.*, **55**, 368 (2003).
17. Y.J. Xue, K.C. Turner, J.B. Meeker, J. Pursley, M. Arnold and S. Unger, *J. Chromatogr. B*, **795**, 215 (2003).
18. L. Deng, F. Wang and H. Li, *Eur. J. Clin. Pharmacol.*, **61**, 831 (2005).

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