

# Pharmacokinetics Study of Pioglitazone (30 mg) Tablets in Healthy Volunteers

Sajal K. Saha<sup>1</sup>, A. K. Azad Chowdhury<sup>1</sup> and Sitesh C. Bachar<sup>2</sup>

<sup>1</sup>Department of Clinical Pharmacy & Pharmacology, University of Dhaka, Dhaka-1000, Bangladesh

<sup>2</sup>Department of Pharmaceutical Technology, University of Dhaka, Dhaka-1000, Bangladesh

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**ABSTRACT:** Pioglitazone, an excellent insulin sensitizer, is used for the treatment of type 2 diabetes mellitus (T2DM). The present investigation demonstrated a single dose pharmacokinetic study of pioglitazone tablet in 24 healthy male volunteers in a randomized protocol. Blood samples were collected before and 0.5 to 24.0 h after a single oral dose of a 30 mg pioglitazone tablet. Plasma pioglitazone level was determined using a validated method of reversed phase binary high performance liquid chromatography (HPLC). The pharmacokinetic parameters determined were  $1.117 \pm 0.315$  ( $\mu\text{g/ml}$ ),  $2.5 \pm 0.735$  (h),  $9.014 \pm 3.385$  ( $\mu\text{g.h/ml}$ ),  $8.081 \pm 3.407$  ( $\mu\text{g. h/ml}$ ),  $1.315 \pm 0.964$  (h),  $0.707 \pm 1.636$  ( $\text{h}^{-1}$ ),  $0.078 \pm 0.02$  ( $\text{h}^{-1}$ ) and  $8.884 \pm 03.808$  (h) for  $C_{\text{max}}$ ,  $T_{\text{max}}$ ,  $AUC_{0-\infty}$ ,  $AUC_{0-24}$ ,  $K_{\text{el}}$ ,  $T_{1/2}(\alpha)$ ,  $K_{\text{el}}$ , and  $T_{1/2}(\beta)$ , respectively. Variation of pioglitazone pharmacokinetic parameters in Bangladeshi population compared to Chinese, Thai and German population may indicate the polymorphic variation in the pioglitazone responsive metabolizing gene CYP3A4 and CYP2C19 in our population.

**Key words:** Pioglitazone hydrochloride, tablet, pharmacokinetics, HPLC

## INTRODUCTION

Type 2 diabetes mellitus has become a global public health problem. Understanding the pathogenesis of diabetes, finding better therapeutic options and ensuring their dose profile, clinical safety and efficacy is the crying need of the time. The environmental conditions under different geographical locations manipulate the genetic characters of the inhabitants living in any particular area. These geographical and genetical influences are characterized by physiological and biochemical expression, which are peculiar to the residents.<sup>1</sup> These differences have important impacts on the pharmacokinetics of different drugs and their responses. Most of the drug metabolizing genes show different responses in different populations, which are often the major determinants of variable drug exposure and response.<sup>2,3</sup> Moreover, individuals, can

differ greatly in their inherent capacity to absorb, distribute, excrete and metabolize drugs. Recent developments in medicine and pharmacology indicate that under certain circumstances the estimation of drug concentration in blood is the best available guide to ascertain dose requirements of patients.<sup>4,5</sup> The absorption, distribution, metabolism and elimination of drugs are highly related to human physiological mechanisms.<sup>6</sup> From the reviewed literature, it was found that no study was conducted in Bangladesh on these aspects. This observation has led to an increased emphasis on the pharmacokinetics of pioglitazone under the indigenous conditions of Bangladesh.

Accomplishment of clinical benefits, safe and effective drug therapy is difficult due to large inter-patient variability in response to many drugs.<sup>3</sup> The knowledge of a drug's pharmacokinetic profile within individual has allowed the clinician to ensure optimization of the drug's performance.<sup>7</sup> Hence, the pharmacokinetic studies are highly relevant for the circumstances under which the drugs are to be used.

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**Correspondence to:** Sajal Kumar Saha  
Tel: +8801720579559; Email: sajal@du.ac.bd

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Traditional treatment strategies include various oral anti-hyperglycemic agents but practically these are not so effective. The thiazolidinediones or glitazones were introduced in the late 1990s for the treatment of type 2 diabetes mellitus.<sup>8</sup>

Pioglitazone is a thiazolidinedione (TZD) derivative of novel oral hypoglycemic agent for the management of type 2 diabetes.<sup>9</sup> It is one of the peroxisome proliferator-activated receptor (PPAR- $\gamma$ ) agonist that increases transcription of insulin-responsive genes and thus increases insulin sensitivity.<sup>10</sup> It leads to regulation of carbohydrate and lipid metabolism as well as adipocyte differentiation. Pioglitazone stimulates the uptake of glucose and fatty acids into cells by promoting the synthesis and expression of cellular glucose and fatty acid transporters. Many studies of pioglitazone demonstrated the improvement of glycemic control, HbA1c, fasting glucose levels and significant decrease in triglycerides and an increase in HDL cholesterol levels, with no overall effect on total cholesterol and LDL.<sup>11</sup> In common with other thiazolidinediones, pioglitazone ameliorates insulin resistance associated with type 2 diabetes without stimulating insulin release from pancreatic  $\beta$  cell, thus lowering the risk of hypoglycemia.<sup>12</sup>

After absorption of pioglitazone from the gastrointestinal tract, peak plasma concentration is observed within two hours.<sup>13</sup> It is rapidly absorbed within an hour and achieve peak concentrations at 2-3 h. The absolute bioavailability ranges between 70-96% with a mean value of 83%.<sup>14</sup> Food slightly delays the time to peak serum concentration 3-4 h but does not alter the extent of absorption.<sup>15</sup> Pioglitazone is highly bound to plasma proteins (>99%) primarily to serum albumin. Pioglitazone is metabolized mainly by CYP3A4 and CYP 2C8/9.<sup>16</sup> Pioglitazone and its metabolites are excreted 15-30% via urine, and the remainders are excreted into bile and feces.<sup>9</sup>

## MATERIALS AND METHODS

**Study subjects.** Twenty four healthy Bangladeshi male adult volunteers [mean age 22.64  $\pm$  2.92, range 20-30 years, mean body weight 63.40  $\pm$

8.25 kg, range 59-70 kg, mean height 163.93  $\pm$  4.95 cm, range 165-186 cm and mean body mass index (BMI) 22.45  $\pm$  1.86] were enrolled in this study. The volunteers were informed of the risks, benefits, procedures and aims of the study as well as their right as research subjects. The study was conducted according to the declaration of Helsinki (1964).<sup>17</sup> Each volunteer signed an informed consent document before entering the study. Ethical permission was taken to approve the protocol and consent form of this study from the ethical committee of Bangladesh Medical Research Council (BMRC/ERC/2007-2010/2024; Date 04, August 2009).

**Reagent and chemicals.** Pioglitazone hydrochloride (99.98% purity), as a reference standard and rosiglitazone (99.96% purity) as an internal standard were purchased from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Sodium dihydrogen phosphate, disodium hydrogen phosphate and glacial acetic acid were of analytical reagent grade. All solvents were of HPLC grade and obtained from Scharlau, Sentmenat, Spain. Ammonium acetate buffer (pH 4.50; 20 mM) was prepared by dissolving 1.52 g of ammonium acetate and 1.3 ml of glacial acetic acid in 800 ml of water. The volume was adjusted to 1000 ml and the pH was set at 4.50 by addition of glacial acetic acid. Purified water was obtained from Centre for Advanced Research in Sciences (CARS), University of Dhaka, Bangladesh. Ibrahim Memorial Diabetes Centre, Dhaka, Bangladesh, provided human plasma samples.

**Chromatographic condition.** The HPLC system consisted of a Shimadzu prominence module with UV-VIS (SPD20AVP, Shimadzu) detector (Shimadzu, Kyoto, Japan). The chromatographic separation was carried out on a Luna C<sub>18</sub> (250 mm  $\times$  4.6 mm) column (Phenomenex, Torrence, CA, USA). The mobile phase was comprised of acetonitrile and 20 mM ammonium acetate buffer pH 4.5  $\pm$  0.2 (60:40 v/v). All separations were performed at a flow rate of 1.0 ml/min. The injection volume was 20  $\mu$ l and the column was maintained at ambient temperature. The peaks were determined using a UV detector set at a wave length of 269 nm.

**Blood collection.** Venous blood samples (6.0 ml) were collected in heparinized tubes immediately before and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 8.0, 12.0 and 24.0 h after drug administration. Plasma was separated by centrifuging the tubes at 1000 relative centrifugal force (RCF) for 20 min. Plasma samples were stored at 20°C until analysis.

**Preparation of stock and standard solutions.** The stock solutions (200 µg/ml) of pioglitazone and the internal standard were prepared separately in dimethylsulfoxide (DMSO). The stock solution was diluted as necessary using the mobile phase as the diluent. Working standard solutions of pioglitazone were prepared by mixing the secondary standard solutions with drug free human plasma to a final pioglitazone concentration of 0, 0.05, 0.10, 0.25, 0.50, 1.0 and 2.0 µg/ml. The working internal standard solution with a concentration of 0.05 µg/ml was prepared by diluting the stock solution with the mobile phase.

**Preparation of sample.** Plasma (1.0 ml) was mixed with 0.25 ml of 0.1 mol/L K<sub>2</sub>HPO<sub>4</sub> in a 10 ml glass tube and the tube was applied on a vortex mixer for 30 sec. Ethyl acetate (5.0 ml) was added and the tube was subjected again to a vortex mixer for 3 min. The tube was centrifuged at 1300 RCF for 6 min. A sample of the supernatant (4.0 ml, exclusively organic phase) was removed to a separate tube and the fluid phase was evaporated by placing the tube in a 45°C water bath under a stream of nitrogen gas. The dried residue was dissolved in 0.1 ml of DMSO. A 20 µl of the solution was injected onto the HPLC column.

## RESULTS AND DISCUSSION

**Linearity.** The calibration curve was linear over the range of 0.05-2.0 µg/ml in human plasma. The linear equation was typically  $Y = 64510 X - 1128.6$ ,  $r^2 = 0.9995$  [ $Y =$  peak area and  $X =$  concentration (µg/ml)]. The recovery was more than 80% and relative standard deviation (RSD) for intra-day and inter-day assay was less than 10%. The limit of quantification (LOQ) was 0.05µg/ml.

**Specificity of the method.** The chromatogram of the pioglitazone in standard and sample were recorded. In the chromatograms of sample, some additional peaks were observed which may be due to presence of plasma constituent. These peaks however, did not interfere with the standard peaks, which demonstrate that the assay method was specific. The results revealed that the peak is free from interference, which shows that the HPLC method is specific.

**Accuracy and precision.** The accuracy and precision (Intra- and inter-day) was ascertained based on quality control samples (QCs) analysis. A result obtained from six replicate injections of the QCs in plasma as summarized in Table1.

**Recovery.** The recovery was examined from QCs for low, medium and high concentration ranges in plasma samples. The recovery was expressed as the percentage of analytes recovered by the assay. In plasma, the average recovery was more than 80%. The high recovery confirmed the suitability of the method for analysis of pioglitazone in the given samples.

Here, we studied the bio-disposition of pioglitazone, using healthy Bangladeshi volunteers as the test subjects (Figure 1). In the present study, the most commonly used two compartmental method was applied with the aid of APO, MWPHARM version 3.02 software for the calculation of pharmacokinetic parameters as shown in Table 2.

The peak plasma concentration ( $C_{max}$ )  $1.117 \pm 0.315$  µg/ml was achieved at time ( $T_{max}$ ) of  $2.514 \pm 0.735$  (h) after oral administration of 30 mg tablet in 24 healthy male volunteers. The value of  $C_{max}$  was almost comparable to the value  $1.329 \pm 0.667$  µg/ml in male volunteers after oral administration of 1.5 times larger (45 mg) dose of pioglitazone.<sup>18</sup> It was less than  $1.598 \pm 0.659$  µg/ml and  $1.359$  ( $1.095$ - $1.623$ ) µg/ml respectively.<sup>19,20</sup> Our calculated ( $C_{max}$ ) values were very close to the value  $1.01$  µg/ml ( $C_{max}$ ).<sup>21</sup> There was significant differences in the peak plasma concentration of Bangladeshi population with the Chinese population having  $1.85$  µg/ml and Thai population having  $2.2$  µg/ml.<sup>22,23</sup> The variation may

be due to metabolizing gene.  $T_{max}$ : The value 2.514 h (1.1-3.8 h) is comparable with the values 2.0 h (1.0 - 4.0 h) and 2.3 h (0.5 - 4.0 h).<sup>18,24</sup> AUC: Mean area under the plasma concentration-time curve ( $AUC_{0-24}$ ) of pioglitazone  $8.081 \pm 3.407 \mu\text{g}\cdot\text{h}/\text{ml}$  was varied with the values 10.89, 8.89, 10.2 and  $11.86 \mu\text{g}\cdot\text{h}/\text{ml}$  cited.<sup>20,21,25</sup> By giving 1.5 times larger dose (45 mg) of pioglitazone, the area founded  $17.387 \mu\text{g}\cdot\text{h}/\text{ml}$  was greater than the area observed in our study.<sup>18</sup>  $T_{1/2}$   $\alpha$ : Absorption half-life of pioglitazone in the present

study was  $1.169 \pm 0.630$  h (0.2924 -2.431 h).  $T_{1/2}$   $\beta$ : Our calculated mean elimination half-life ( $8.83 \pm 3.808$  h) was less as compared to the value of mean elimination half-life of pioglitazone,  $13.7 \pm 5.7$  h and  $9.818$  h respectively.<sup>18,19</sup>  $V_d$ : Apparent volume of distribution  $31.077 \pm 14.529$  L ( $0.506 \pm 0.44$  L/Kg) of pioglitazone observed was comparable to the volume of distribution  $0.63 \pm 0.41$  L/kg as cited in Budde *et al.*<sup>19</sup>

**Table 1. Intra- and inter- day precision and accuracy of HPLC assay of pioglitazone in plasma (n = 6, each day).**

$C_{\text{added}}$ ( $\mu\text{g}/\text{ml}$ )	Intra-day			Inter-day		
	$C_{\text{obs}}$ ( $\mu\text{g}/\text{ml}$ )	RSD	Recovery %	$C_{\text{obs}}$ ( $\mu\text{g}/\text{ml}$ )	RSD	Recovery %
0.05	$0.043 \pm 0.01$	1.98	80.75	$0.039 \pm 0.01$	2.56	79.28
0.1	$0.031 \pm 0.01$	1.76	82.10	$0.080 \pm 0.01$	1.74	80.36
0.5	$0.40 \pm 0.02$	2.20	80.64	$0.39 \pm 0.01$	1.62	79.53

**Table 2. Pharmacokinetic parameters derived after a single oral dose of 30 mg pioglitazone tablet (arithmetic mean  $\pm$  SD, n = 24).**

Pharmacokinetic parameters	Mean	$\pm$ SD	Min	Max
Area under the curve ( $AUC_{0-\infty}$ ) [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ]	9.014	3.835	4.073	18.85
Area under the curve ( $AUC_{0-24}$ ) [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ]	8.081	3.407	4.055	11.42
Peak concentration $C_{\text{max}}$ [ $\mu\text{g}/\text{ml}$ ]	1.117	0.315	0.756	1.986
Time to peak concentration $T_{\text{max}}$ [h]	2.514	0.735	1.108	3.879
Absorption rate constant ( $k_a$ ) [1/h]	0.707	1.636	0.266	1.653
Absorption half-life [h]	1.315	0.964	0.101	3.694
Elimination rate constant ( $K_{\text{el}}$ ) [1/h]	0.078	0.268	0.145	0.615
Elimination half-life ( $T_{1/2}$ ) [h]	8.088	3.808	1.761	13.87
Volume of distribution ( $V_d$ ) [l]	31.07	14.529	12.86	54.79
Mean residence time (MRT) [h]	9.426	3.358	4.358	13.41
Lag-time ( $T_0$ ) [h]	0.377	0.494	0.084	1.912

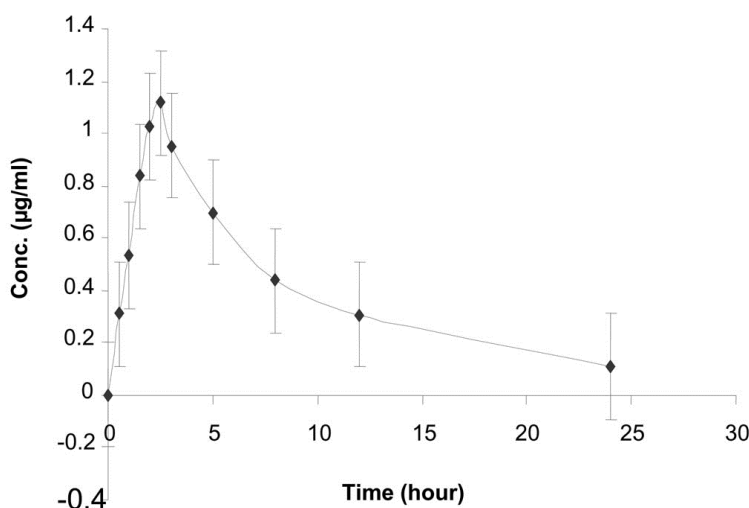


Figure 1. Mean plasma concentration time curve derived after a single oral administration of 30 mg pioglitazone tablet.

**Table 3. Overview of the comparison of pharmacokinetics of pioglitazone with foreign counterparts.**<sup>22, 23</sup>

Pharmacokinetic parameters	Healthy population				
	Bangladeshi	Pakistani	Chinese	Thai	Germany
AUC <sub>0-∞</sub> [µg. h/ml]	9.014 ± 3.835	7.71 ± 2.63	16.27 ± 4.18	29.446 ± 24.97	17.387 ± 3.68
AUC <sub>0-24</sub> [µg. h/ml]	8.081 ± 3.407	6.56 ± 2.11	15.51 ± 4.22	26.128 ± 17.02	14.56 ± 5.320
C <sub>max</sub> [µg/ml]	1.117 ± 0.315	0.86 ± 0.156	1.85 ± 0.58	2.265 ± 8.64	1.329 ± 0.965
T <sub>max</sub> [h]	2.514 ± 0.735	2.40 ± 0.462	1.80 ± 0.6	1.95 ± 0.00	2.0 ± 0.532
T <sub>1/2</sub> [h]	8.884 ± 3.808	8.83 ± 2.823	8.6 ± 0.9	9.818 ± 0.00	13.7 ± 2.511

Success of a drug and a drug delivery system depend on the fate of the drug in the human body.<sup>6</sup> Many studies have identified inappropriate pharmacokinetics as the major cause for the failure of a drug development. Appropriate consideration regarding the safety and efficacy of a drug requires an analysis of the links between systemic exposure and effects of the drug. Hereditary differences affect drug metabolism and thus play as a major determinant of variable drug exposure and response.<sup>3</sup> However, many drugs are developed, tested and formulated in one country and then exported and used in another. Beside genetic differences, environmental differences may also play important roles in drug metabolism, exposure and response. Therefore, there is a need for studying the bio-disposition of drugs in the target population residing in diverse environments.

Pharmacokinetic parameters of pioglitazone on different population has been depicted in table 3. Somruedee et al.<sup>23</sup> examined similar parameters on Thai population and observed C<sub>max</sub> of 1.14 ± 0.29 µg/ml at the t<sub>max</sub> of 2.00 ± 1.61 h. Budde<sup>18</sup> used a single dose of 45 mg pioglitazone and observed a C<sub>max</sub> 1.329 ± 0.667 µg/ml at the t<sub>max</sub> of 2.0 h (1.0-4.0 h). These values are comparable to the values we observed for Bangladeshi population. However, Hua et al.<sup>21</sup> observed a C<sub>max</sub> 1.85 µg/ml at the t<sub>max</sub> of 1.80 ± 0.6 h using the same dose on Chinese population. These observations reflect that there is indeed difference in pioglitazone metabolism among different ethnic groups. In Bangladeshi population, the pharmacokinetic parameters of pioglitazone (30 mg tablet) were found to vary significantly with Western, Chinese and Thai population and showed similarities with Indian and Pakistani population. This similarities and significant differences may be

attributed to various genetic and environmental factors. Moreover, these differences may depend to same extent upon the differences between the analytical methods used.

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## REFERENCES

- Nawaz, M. and Shah, B.H. 1985. Geometrical considerations in the quality assurance of pharmaceuticals. *Proc. Int. Seminar on Quality Assurance of Pharmaceuticals, Karachi, Pakistan*, pp. 311-320.
- Flockhart, D.A. and Desta, Z. 2009. Pharmacogenetics of drug metabolism. *Clin. Trans. Sci.* **9**, 301-317.
- Vesell, E.S. and Passananti, G.T. 1971. Utility of clinical chemical determinations of drug concentrations in biological fluids. *Clin. Chem.* **17**, 851-866.
- Vesell, E.S. 1974. Relationship between drug distribution and therapeutic effects in man. *Annu. Rev. Pharmacol.* **14**, 249-270.
- Tsuchida, E. and Abe, K. 1982. Interactions between macromolecules in solutions and intermolecular complexes. *Adv. Polym. Sci.* **45**, 1-119.
- Smith, D.A., Jones, B.C. and Walker, D.K. 1996. Design of drugs involving the concepts and theories of drug metabolism and pharmacokinetics. *Med. Res. Rev.* **16**, 243-266.
- Gale, E.A. 2001. Lessons from the glitazones: a story of drug development. *Lancet* **357**, 1870-1875.
- Aronof, S., Rosenblatt, S., Egan, J.W. and Mathisen, A.L. 2000. Pioglitazone hydrochloride monotherapy improves

- glycemic control in the treatment of patients with type 2 diabetes, a 6 month randomized placebo controlled dose response study. The pioglitazone 001 study group. *Diabetes Care* **85**, 1605-1611.
9. Gilis, P.S. and Dunn, C.J. 2000. Pioglitazone. *Drugs* **60**, 333-43.
  10. Dormandy, J., Charbonnel, B. and Eckland, D. 2005. Secondary prevention of macro vascular events in patients with type 2 diabetes in the PRO active study (prospective pioglitazone clinical trial in macro vascular events) a randomized controlled trial. *Lancet* **366**, 1279-1289.
  11. Madan, P. 2005. Effect of thiazolidinediones on lipid profile. *Canadian. Med. Assoc. J.* **173**, 344-5.
  12. Eckland, D.A. and Danhof, M. 2000. Clinical pharmacokinetics of pioglitazone. *Exp. Clin. Endocrinol. Diabetes* **108**, S234-S242.
  13. Baba, S. 2001. Pioglitazone: A reviews of Japanese clinical studies. *Curr. Med. Res. Opin.* **17**, 166-189.
  14. Geerlof, J.S. Lebrizzi, R. and Carey, R.A. 2000. Effect of food on the pharmacokinetics of pioglitazone. *Diabetes* **49**, Suppl. 1-A357.
  15. Inzucchi, S.E. 2002. Oral antihyperglycemic therapy for type 2 Diabetes: *Scientific review. J. Am. Med. Assoc.* **287**, 360-72.
  16. Budde, K., Neumayer, H., Fritsche, L., Sulowicz, W., Stompor, T. and Eckland, D. 2003. The pharmacokinetics of pioglitazone in patients with impaired renal function. *Br. J. Clin. Pharmacol.* **55**, 368-374.
  17. Joseph, H., Brenda, E. and Lerner, K. 2012. Helsinki Declaration 1964. *Biotechnology* **2**, 483-485.
  18. Sripalakit, P.S., Maphanta, P., Neamhom, A., Saraphanchotiwitthaya, S., Polno, k. and Yokubol, D. 2007. Comparative study on the bioequivalence of two formulations of Pioglitazone tablet in healthy Thai male volunteers. *J. Drug Dev. Ind. Pharm.* **33**, 1362-1368.
  19. Deng, L.F. Wang and Li, H. 2005. Effect of gemfibrozil on the pharmacokinetics of pioglitazone. *Eur. J. Clin. Pharmacol.* **61**, 831-836.
  20. Wong, H., Ozalp, Y., Lainesse, A. and Alpan, R.S. 2004. *In-vivo* bioequivalence of oral anti-diabetic agents: pioglitazone tablets. *Arzneimittelforschung.* **54**, 618-624.
  21. Hua, Z., Xue-Qing, W., Xuan, Z., Qiang, Z., Qi, Y. and Ke-Xin, Li. 2004. Study on bioequivalence of pioglitazone hydrochloride tablets in healthy Chinese volunteers. *Chinese J. Chromatogr.* **4**, 119-122.
  22. Somruedee, C. 2000. Bioequivalence study of 30 mg pioglitazone tablets in thai healthy vounteers. *J. Med. Assoc. Thai.* **90**, 564-8.
  23. Xue, Y.J., Turner, K.C., Meeker, J.B., Pursley, J., Arnold, M. and Unger, S. 2003. Quantitative determination of pioglitazone in human serum by direct-injection high-performance liquid chromatography mass spectrometry and its application to a bioequivalence study. *J. Chromatogr. B.* **795**, 215-226.
  24. Yanwen, Z., Festival, L.S., Yang, Y. and Jian, L. 2004. The relative absorption of pioglitazone hydrochloride capsules in healthy volunteers. *Chinese J. Hosp. Pharm.* **24**, 598-600.
  25. Food and Drug Administration (FDA). 2007. *New Drug Applications. NDA 21-925/S-004.*