Pioglitazone HCl Levels and Its Pharmacokinetic Application in Presence of Sucralose in Animals Serum by HPLC Method

Lina Tamimi¹, Wael Abu Dayyih*¹*, Nidal Qinna², Eyad Mallah¹ and Tawfiq Arafat¹

¹Department of Pharmaceutical Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy and Medical Sciences-University of Petra, Jordan
²Department of Pharmacology and Biomedical Sciences-University of Petra, Jordan

Abstract

Purpose: to develop a simple, valid and rapid chromatographic method for quantifying pioglitazone HCl in rats serum in order to study the pharmacokinetic parameters of pioglitazone HCl in rats serum fed with sucralose simultaneously for examination of interaction possibility between pioglitazone HCl and sucralose in rats.

Methods: In our developed method of analysis, mobile phase was consisted of [51.50%] acetonitrile and [48.50%] 0.025 mM ammonium acetate with pH of 8, column of separation was C8 at temperature of 40°C using injection volume of 90 µl, mobile phase flow rate was 1 ml/min and samples run time was 10 min, the signals were monitored and analyzed at λ=269 nm and sildenafil citrate was used as internal standard. Pioglitazone was given to rats orally of [10mg/kg] dose while sucralose was given with [11 mg/kg/day] dose.

Results: A successful HPLC method was validated and developed to quantify pioglitazone HCl in rats serum, overall intra-day precision and accuracy were reasonable with CV % values range [0.16-3.54] and accuracy % range [98.4-107.9], while inter-day precision and accuracy showed accepted precision with CV% range [0.15-4.13] and accuracy % range [99.35-103.99]. The coefficient of correlation was 0.9991 with reasonable sensitivity and selectivity. Combination effect of pioglitazone with sucralose on pioglitazone serum profile was demonstrated as strong statistical effect according to Cohen’s d and significant P values too.

Conclusion: A successful HPLC method was validated and developed to quantify pioglitazone HCl in rats serum, combination effect of pioglitazone with sucralose over all time intervals of pioglitazone serum profile was demonstrated as strong statistical effect.

Keywords: HPLC; Pioglitazone; Sucralose; Pharmacokinetic; Interaction

Abbreviations: HPLC: High performance liquid chromatography; QC: Quality control; EMEA: European medicines agency; LLOQ: Lower limit of quantification; CQL: Quality control low; QC M: Quality control medium; QC H: Quality control high; CYP: cytochrome p; CV%: coefficient of variation; AUC: area under the curve; Cmax: maximum concentration; Tmax: maximum time at Cmax; r²: correlation coefficient; IS: internal standard; PG: pioglitazone; HR: hour; ST: standard; NA: not available

Introduction

Pioglitazone: [±]-5-[4-[2-[5-ethyl-2-pyridinyl] ethoxy] phenyl methyl]-2, 4]-thiazolidinedione monohydrochloride [1], the structural formula is as shown in Figure 1.

It belongs to a different chemical class with different pharmacological action than the sulfonylureas, metformin, or the α-glucosidase inhibitors [2], it is a compound that belongs to a group named “thiazolidinediones” family, an oral anti-diabetic agent that acts by decreasing insulin resistance [3]. Pioglitazone has the same mechanism of action by which all thiazolidinediones act inside the body. Its mechanism of action made it as one of the most effective drugs that is used in the management of type 2 diabetes mellitus [4,5], pioglitazone can improve the sensitivity to insulin in muscle and adipose tissue [2,6,7] which helps in hepatic gluconeogenesis inhibition [8] and improves glycemic control by reducing circulating insulin levels, [9-11].

Type 2 diabetes patients are usually treated with numerous pharmaco logical compounds which increases the susceptibility to be exposed to risky drug-drug interactions.

Pioglitazone was marketed at USA in 1999, nowadays; it is marketed in more than 40 countries worldwide [6].

Pioglitazone HCl is extensively metabolized by hydroxylation and oxidation via liver CYP450 enzymatic system [12], in vitro data emphasized that multiple CYP isoforms are involved in pioglitazone

![Figure 1: Pioglitazone hydrochloride chemical structure.](image-url)
Sucralose is a synthetic organochlorine sweetener, with chemical formula of Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside [15], the chemical structure is shown in Figure 2. It is considered as one of the most common sweeteners in the world’s food industries [16] and pharmaceutical manufacturing [17].

Sucralose is a derivative of the halogenated sucrose, mellow, with aromatic flavor and good stability, low in calories [around 3 calories per 1 gm], there are significant gaps in our current knowledge concerning pharmacokinetics of these sweeteners and their potential for sweetener–drug interactions. Recently, previous studies have approved that sucralose increases the expression of the P-glycoprotein intestinal transporter and induces CYP3A4 enzyme activity in intestine and liver [18,19] at levels that have been associated with reduced bioavailability, pharmacokinetic and pharmacodynamic parameters of drug, it may occur if the drug is to be metabolized by CYP3A4 enzyme and concurrently taken with sucralose at doses that are approved by the FDA, these findings justify the need of further studies concerning potential sucralose-drug interactions [20].

A new developed, simple, valid and rapid high performance liquid chromatography-ultraviolet method according to EMEA guidelines for quantification of pioglitazone in rats serum in presence and absence of sucralose to determine any possible interaction between both substances through the effect on pioglitazone serum levels during pioglitazone serum levels time profile [21].

**Materials and Methods**

**Chemicals**

HPLC/UV is a technique of analysis that could be used for determination of several compounds in plasma or serum [22,23]. Methanol and acetonitrile HPLC gradient were used for HPLC analysis [FULLTIME, USA], deionized water HPLC gradient and ammonium acetate HPLC gradient were obtained from [TEDIA, USA], Ammonia solution was obtained from [JPM, Jordan] while pioglitazone hydrochloride raw material was from [YASHICA pharmaceuticals, India], while sucralose was acquired from its finished product Splenda®. Intra-day precision and accuracy were evaluated by analyzing the same HPLC conditions for each calibration curve, peak area ratios were calculated and the correlation coefficient $r^2$ was accepted if it is more than 0.98.

**Apparatus and chromatographic conditions**

The HPLC system [HITACHI, Japan] consisted of S# L-2130 VWR-HITACHI pump model, S# L-2200 VWR-HITACHI autosampler thermostat, S# L-2300 VWR-HITACHI column oven and S# L-2420 VWR-HITACHI UV detector.

Chromatographic separation was carried out using mobile phase consisted of [51.50%] acetonitrile and [48.50%] 0.025 mM ammonium acetate with pH of 8, column of separation was [ACE C8, 5 µm [250×4.6 mm i.d.]] at temperature of 40°C using injection volume of 90 µl, mobile phase flow rate was 1 ml/min and samples run time was 10 min, the signals were monitored and analyzed at $\lambda = 269$ nm and sildenafil citrate was used as internal standard.

**Preparation of stock and working solutions**

Pioglitazone HCl oral dose of [10 mg/kg/day] was recommended for rats and prepared by dissolving the drug in 0.0375 M of NaOH solution while sucralose oral dose of [11 mg/kg/day] was prepared as a working solution of [250 mg/ml] Splenda®, sucralose solution was prepared by dissolving Splenda® powder with distilled water at room temperature under sonication, both working solutions were prepared freshly and directly before experiment and kept away of heat and light.

Pioglitazone hydrochloride stock solution was prepared by dissolving [25 mg] in 50 ml methanol to get a stock solution of 500 µg/ml while pioglitazone working solutions were prepared from the stock solution by dilution with methanol directly before use and kept away of light and heat sources, sildenafil citrate was used as internal standard. Precision and accuracy

To examine and evaluate the precision and accuracy of analysis method, four different concentrations were prepared and used; lower limit of quantification, low, middle and high concentrations of pioglitazone by spiking with rats serum.

Intra-day precision and accuracy were evaluated by analyzing the same HPLC conditions for each calibration curve, peak area ratios were calculated and the correlation coefficient $r^2$ was accepted if it is more than 0.98.

**Calibration samples**

Serial dilutions of samples were prepared by taking an appropriate volume of each working concentration that is previously prepared in methanol with sufficient volume of rats serum was added to reach 5 ml final volume with corresponding calibration concentration. Samples were vortexed, then used for analysis procedure, all final standard solutions were prepared freshly and directly before analysis.

Six different calibration concentrations were analyzed under the same HPLC conditions for each calibration curve, peak area ratios were calculated and the correlation coefficient $r^2$ was accepted if it is more than 0.98.

**Sample preparation [extraction procedure]**

Mixing of 100 µl of rats serum with 75.0 µl of IS working solution, then vortex for 30 seconds and centrifuged at 12000 rpm for 5 min. Supernatant was transferred into rack, and 90 µl of supernatant was injected into HPLC unit.

**Stability**

Room temperature stability: three samples of [QCL and QCH] of concentrations [2 and 12.8 µg/ml] were prepared and analyzed with freshly prepared calibration at zero time and after 8 hours at room temperature of 28°C. Area ratios % per QCL and QCH were calculated referring to related calibration.

Freeze and thaw stability: Three samples of [QCL and QCH] were prepared properly in serum with sufficient final volume covering all test cycles analysis.

At zero time: with corresponding calibration, samples were analyzed and concentrations were calculated, then, samples were stored and frozen at -20°C.

After 12 hours: samples were thawed at room temperature 25°C which was the samples processing temperature during analysis stages. After complete thawing, samples were analyzed again with...
corresponding calibration, and refrozen again for 24 hours.

After 24 hours: samples were thawed and analyzed under same conditions. After each cycle, concentrations were calculated referring to related calibration.

Long term stability: three samples of [QCL and QCH] were analyzed with freshly prepared calibration at zero time. Samples then were frozen for 30 days at temperature of -20°C. After long term freezing period was finished, samples were removed and thawed at room temperature.

Room temperature and long term stability test were also carried out for working solutions, stability test was assessed by comparing the calculated concentrations with the nominated concentrations as it should be within ± 15% for both QCL and QCH.

Preclinical Study

The study protocol was approved by the Research Committee [October; 5/10/2013] at the Faculty of Pharmacy, University of Petra, Amman, Jordan.

Adult male Sprague Dawley laboratory rats were supplied at University of Petra Animal House. Rats average weight was [0.230 kg ± 0.03]. Rats were placed in air- conditioned environment with temperature of [20-25°C] and exposed to a photoperiod cycle [12 hour light /12 hour dark] with humidity of 50% daily. Rats were under fasting for 24 hours, and weighed directly before the experiment. All used rats were in healthy conditions before and after experiment as rats were monitored for one month post analysis.

Pioglitazone HCl and sucralose test solutions were freshly prepared directly in the laboratory before rats feeding in order to avoid any possible decomposition of either pioglitazone HCl or sucralose.

A group consisting of total 80 healthy rats was used for the experiment. Rats were divided into groups of 8 rats, rats then were weighed and numbered orderly. Pioglitazone HCl and sucralose oral doses were calculated according to each rat weight then given orally according to ordered numbers using gastric gavage. Trials analysis was performed among 3 days according to following arrangement:

At first day of trials: two groups of rats were used for analysis ; the first one has received water at experiment zero time, then followed by pioglitazone HCl after 1 hour of water feeding, while the second group has received sucralose at zero time of experiment followed by pioglitazone HCl after 1 hour of sucralose feeding. Time intervals of blood samples pooling were: 0, 30 min, 1 hr, 2 hr, 3 hr, 4 hr and 6 hr.

At second day of trials: two groups have received water at zero time of experiment followed by pioglitazone after one hour, while other two groups have received sucralose at zero time followed by pioglitazone after 1 hour of sucralose feeding. Time intervals of blood samples pooling were: 0, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr and 24 hr.

At third day of trials: one group has received water at zero time of experiment followed by pioglitazone after one hour, while another three groups have received sucralose at zero time followed by pioglitazone after 1 hour of sucralose feeding. Time intervals of blood samples pooling were: 0, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr and 8 hr.

Tail tip of each rat was cut after weighing and numbering, approximately 200 µl of blood was pooled into eppendorf tube at each time interval under the same numbering order, after total time intervals of blood pooling is finished, samples were centrifuged for 10 minutes [12000 rpm] in order to obtain pure serum that is needed for analysis then frozen at -20°C.

Results

Acceptable separation of pioglitazone and sildenafil citrate in serum was obtained as the pioglitazone peaks were sharp with no tailing or splitting, reasonable retention time and clear chromatograms, as mentioned in Figures 3-6 which represents an overlay chromatograms for blank rats serum, rats serum with IS, rats serum with LLOQ, and rats serum with IS and pioglitazone after 30 minutes of combination respectively.

Precision, accuracy and linearity were measured to estimate the method performance.

Linearity calibration curves were designed with six different trials, the correlation coefficient and calibrations equations were mentioned in Table 1.

High correlation coefficient indicated the reasonability of linearity between the peak area ratio of pioglitazone in serum and the quality control concentrations used in calibrations.

Inter-day and intra-day precision showed acceptable values as LLOQ the lower limit of quantification showed coefficient of variation [CV%] less than 20% while QCL, QCM and QCH represented
coefficient of variation values less than 15%, inter-day and intra-day results mentioned in Table 2.

On the other hand, Inter-day and intra-day accuracy showed reasonable values as mean calculated concentration of LLOQ was within ± 20% of nominated concentration while it was within ± 15% for QCL, QCM and QCH.

Three different stability tests were designed and carried out to assess the samples stability before and after analysis under all possible conditions that could be kept under via over all experimental phases.

Freeze and thaw stability: data were obtained after each cycle of analysis for two QC concentrations; QCL and QCH, samples were analyzed at zero time then after 12 hours of freezing, samples were thawed for 3 hours then analyzed and finally after another 24 hours of freezing and thawing. All results were listed in Table 2.

Room temperature stability: serum samples of both QCL and QCH were analyzed at zero times and after 8 hours of standing at room temperature of [28°C ± 1], data was mentioned in Table 3.

Long term freezing stability test was carried out as serum samples of both QCL and QCH were analyzed at zero time and after freezing for 30 days, data was collected in Table 4.

Sucralose–pioglitazone HCl combination effect on pioglitazone HCl serum levels: rats serum levels of pioglitazone HCl were calculated through serum samples analysis that obtained from rats groups per day of trials.

| Sample ID | Mean | SD | CV% | Error | Accuracy % |
|-----------|------|----|-----|-------|------------|
| ST[LLOQ]  | 0.26 | 0.01| 1.77| 0.01  | 102.8      |
| QCL       | 1.989| 0.004|0.188| -0.01 | 99.45      |
| QCM       | 7.16 | 0.09| 1.28| 0.16  | 102.3      |
| QCH       | 13.52| 0.11| 0.81| 0.72  | 105.6      |

Table 1: Linearly calibration curves data.

| Calibration no. | Calibration equation | Correlation coefficient [r²] value |
|-----------------|----------------------|----------------------------------|
| Calibration 1   | \( y = 0.100824 x - 0.000986181 \) | 0.997383 |
| Calibration 2   | \( y = 0.103200 x + 0.000412951 \) | 0.994147 |
| Calibration 3   | \( y=0.0985494 x - 0.000155154 \) | 0.999127 |
| Calibration 4   | \( y = 0.101335 x - 0.00541304 \) | 0.996554 |
| Calibration 5   | \( y = 0.0987729 x + 0.00250227 \) | 0.99955 |
| Calibration 6   | \( y = 0.0966408 x - 0.0106092 \) | 0.996124 |

Table 1: Inter-day and intra-day precision and accuracy.

| QC Low [2 µg/ml] | Time [hour] | Mean | Accuracy% | Stability % |
|------------------|-------------|------|-----------|-------------|
| 0 hour           | 2.10        | 110.5|           |             |
| 8 hours          | 2.03        | 105  |           |             |
| QC High [12.8 µg/ml] | Time [hour] | Mean | Accuracy% | Stability % |
| 0 hour           | 12.79       | 100  |           |             |
| 8 hour           | 12.73       | 100  |           |             |

Table 2a: Inter-day and intra-day precision and accuracy.

| QC Low [2 µg/ml] | Time [hour] | Mean | Accuracy% | Stability % |
|------------------|-------------|------|-----------|-------------|
| 0 hour           | 2.03        | 105  |           |             |
| 12 hours         | 1.99        | 99.95|           | 98          |
| 24 hours         | 1.98        | 99.95|           | 97.5        |
| QC High [12.8 µg/ml] | Time [hour] | Mean | Accuracy% | Stability % |
| 0 hour           | 12.75       | 99.68|           |             |
| 12 hours         | 12.61       | 96.13|           | 98.9        |
| 24 hours         | 12.58       | 98.28|           | 98.7        |

Table 2b: Freeze and Thaw Stability Data.
Concentrations values treated statistically to evaluate the effect size and significance of combination between pioglitazone and sucralose.

Cohen’s d value was used for effect size evaluation of combination while P value was used for estimation of combination significance.

Combination effect size values were exceeding 0.8 for all intervals except at 30 minutes as it was small, combination effect significance was strong as all P values were less than 0.05.

At first day of trials the samples were collected starting by zero time and ending by 6 hours as last time interval, (Figure 7), samples were analyzed and data collected properly as in Table 5.

At second day of trials the samples were collected starting by zero time and ending by 24 hours as last time interval, (Figure 8), samples were analyzed and data collected properly as in Table 6.

At third day of trials the samples were collected starting by zero time and ending by 8 hours as last time interval, (Figure 9), samples were analyzed and data collected properly as in Table 7.

Three days of trials results were collected with overall calculated serum concentrations profiles and corresponding AUC values (area under the curve), Cmax and Tmax were summarized in Table 8, with statistical results, (Figure 10).

**Figure 4:** Serum with IS chromatogram [IS: Peak 2].

**Figure 5:** Pioglitazone LLOQ chromatogram [Peak 1 for Pioglitazone HCl, Peak 2 for IS].

**Figure 6:** Pioglitazone HCl unknown concentration chromatogram after 30 minutes oral administration [Pioglitazone HCl: Peak 1, IS: Peak 2].

**Figure 7:** First day trial serum–time profile curve.

**Figure 8:** Second day trial serum–time profile curve.
Discussion

Method validation was evaluated referring to EMEA guidelines:

Precision and accuracy results were reasonable as CV% values and mean calculated concentrations were within range of acceptance.

Linearity: all correlation coefficients were over 0.98

Stability: three stability tests showed reasonable results which confirms method stability.

Sucralose–pioglitazone combination effect: statistical analysis results showed a significant combination interaction between pioglitazone and sucralose when both compounds are given concurrently, as P values represented a strong combination effect which was less than 0.05.

This combination interaction could be justified by sucralose induction effect on CYP 450 enzymes, specifically 3A4 subtype by which pioglitazone is extensively metabolized.

Pioglitazone is basically absorbed in stomach where CYP3A4 isoenzymes are located profusely in parietal cells endoplasmic reticulum, this enzyme will exert its metabolic biotransformational effect over pioglitazone once absorbed, induction effect of sucralose over the CYP3A4 metabolic enzyme will also activate the metabolism of pioglitazone in liver too, which results in pioglitazone plasma/serum levels reduction and over production of pioglitazone active and inactive metabolites.

In rats, CYP3A1, 3A2, 3A9, 3A18, 3A23 and 3A62 have been reported as CYP3A forms. CYP3A23 was classified as identical to CYP3A1 by the analysis of its gene.
CYP3A62 form has been identified as a new rat CYP3A isoenzyme with expression profile similar to human CYP3A4 and rat CYP3A9. CYP3A62 is a predominant form in the intestinal tract, where CYP3A1 and -3A2 were found only in liver [24].

As recent studies illustrated a distinctive binding affinity variance of pioglitazone to reactive sites of CYP3A enzymes during metabolic reactions which justifies its variable bioavailability between humans and rats, this variance impresses a possible perceptible clinical differences in pioglitazone human plasma levels when combined with sucralose, which strongly recommends further clinical research in human.

**Conclusion**

A successful HPLC method was validated and developed to quantify pioglitazone HCl in rats serum, the method was precise and accurate with rational linearity performance and reasonable sensitivity and selectivity.

Concerning stability and recovery tests, all obtained results were reasonable and accepted according to EMEA guidelines.

Combination effect of pioglitazone with sucralose over all time intervals of pioglitazone serum profile was demonstrated as strong statistical effect according to Cohen’s d and significant P values too.

Cmax showed a significant change between presence and absence of sucralose while Tmax didn’t show any change, which suggests the possibility of interaction between pioglitazone HCl and sucralose during combination.

Advanced clinical research on human volunteers to make more precise results concerning pioglitazone HCl–sucralose combination interaction is suggested through the detection and quantification of pioglitazone HCl and its active metabolites as these metabolites are also pharmacoologically active in human body of diabetic patient [20,21].

**Acknowledgment**

The authors would like to thank Faculty of Pharmacy and Medical Sciences at University of Petra.

**References**

1. Radhika B, Vijayakumar S, Dhanpal R (2012) A Pharmacokinetic Interaction of Pioglitazone HCl and Its Clinical Applications: A Short Review 2: 1-9.
2. Smith U (2001) Pioglitazone: mechanism of action. Int J Clin Pract Suppl: 13-16.
3. Nissen SE, Wolski K (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 356: 2457-2471.
4. Grossman LD (2002) New solutions for type 2 diabetes mellitus: the role of pioglitazone. Pharmacoeconomics 20 Suppl 1: 1-9.
5. Chilcot J, Tappenden P, Jones ML, Wight JP (2001) A systematic review of the clinical effectiveness of pioglitazone in the treatment of type 2 diabetes mellitus. Clin Ther 23: 1792-1823.
6. Sohda T, Kawamatsu Y, Fujita T, Meguro K, Ikeda H (2002) Discovery and development of a new insulin sensitizing agent, pioglitazone. Yakugaku Zasshi 122: 909-918.
7. Pavo I, Jeremendi G, Varkonyi T, Kerenyi T, Gyimesi Z (2003) Effect of Pioglitazone HCl compared with Metformin on glycemic control and indicators of insulin sensitivity in recently diagnosed patients with type 2 diabetes. Journal of Clinical Endocrinology & Metabolism 88: 1537-1545.
8. Tan MH (2000) Current treatment of insulin resistance in type 2 diabetes mellitus. Int J Clin Pract Suppl: 54-62.
9. Haffner SM, D’Agostino R, Mykkänen L, Tracy R, Howard B et al. (1999) Insulin sensitivity in subjects with type 2 diabetes. Relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. Diabetes care 22: 562-568.
10. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL (2000) Pioglitazone HCl 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 HCl 001 Study Group. Diabetes care 23: 1605-1611.
11. Richter B, Bandeira-Echtler E, Bergerhoff K, Clar C, Ebrahim SH (2006) Pioglitazone for type 2 diabetes mellitus. Cochrane Database Syst Rev: CD005060.
12. Tan MH (2000) Current treatment of insulin resistance in type 2 diabetes mellitus. Int J Clin Pract Suppl: 54-62.
13. Tornio A, Niemi M, Neuvonen PJ, Backman JT (2008) Tinrethopirin and the CYP2C8*3 allele have opposite effects on the pharmacokinetics of pioglitazone. Drug Metab Dispos 36: 73-80.
14. Holstein A, Beil W, Kovacs P (2012) CYP2C metabolism of oral antidiabetic drugs–impact on pharmacokinetics, pharmacodynamics and pharmacogenetic aspects. Expert Opin Drug Metab Toxicol 8: 1549-1563.
15. Mann SW, Yuschak MM, Amyes SJ, Aughton P, Finn JP (2000) A combined chronic toxicity/carcinogenicity study of sucralose in Sprague-Dawley rats. Food Chem Toxicol 38 Suppl 2: S71-89.
16. Broderick KB, Campbell AA, Meyers MA, Song JH, Yatka RJ et al. [1992] U.S. Patent No. 5,139,796. Washington, DC: U.S. Patent and Trademark Office.
17. Blase CM, Shah MN (1993) U.S. Patent No. 5,272,137. Washington, DC: U.S. Patent and Trademark Office?
18. Schiffman SS, Rothier KI (2013) Sucralose, A Synthetic Organochlorine Sweetener: Overview Of Biological Issues. J Toxicol Environ Health Part B 16: 398-451.
19. Abou-Donia MB, El-Masyr EM, Abdel-Rahman AA, McLendon RE, Schiffman SS (2008) Splenda alters gut microflora and increases intestinal p-glycoprotein and cytochrome p-450 in male rats. J Toxicol Environ Health A 71: 1415-1429.
20. Tanis SP, Parker TT, Colca JR, Fisher RM, Kletzein RF (1996) Synthesis and biological activity of metabolites of the antidiabetic, antihyperglycemic agent Pioglitazone HCl. J of med chem 39:5053-5063.
21. Scheen AJ (2007) Pharmacokinetic interactions with thiazolidinediones. Clin Pharmacokinet 46: 1-12.
22. Noobaran M, Keyhanfar F, Motevalian M, Mahmoudian M (2010) Improved HPLC method for determination of four PPIs, omeprazole, pantoprazole, lansoprazole and rabeprazole in human plasma. J Pharm Pharm Sci 13: 1-10.
23. Watanabe M, Kobayashi M, Ogura J, Takahashi N, Yamaguchi H, et al. (2014) Alteration of pharmacokinetics of grepafloxacin in type 2 diabetic rats. J Pharm Sci 127: 25-33.
24. Matusbara T, Kim HJ, Miyata M, Shimada M, Nagata K, et al. (2004) Isoxazolidinedione and characterization of a new major intestinal CYP3A form, CYP3A62, in the rat. J Pharmacol Exp Ther 309: 1282-1290.