Simultaneous determination of pioglitazone and candesartan in human plasma by LC-MS/MS and its application to a human pharmacokinetic study

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Abstract A simple and rapid liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method has been developed and fully validated for simultaneous quantification of pioglitazone and candesartan in human plasma. Irbesartan was used as an internal standard. The analytes were extracted from human plasma samples by solid-phase extraction technique using a Strata-X 33 μm polymeric sorbent. The reconstituted samples were chromatographed on a C\textsubscript{18} column by using a 80:20 (v/v) mixture of acetonitrile and 0.1% formic acid as the mobile phase at a flow rate of 0.8 mL/min. The calibration curves obtained were linear (r ≥ 0.99) over the concentration range of 15–3000 ng/mL for pioglitazone and 5–608 ng/mL for candesartan. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. A run time of 2.7 min for each sample made it possible to analyze more than 300 plasma samples per day. The proposed method was found to be applicable to clinical studies.

1. Introduction

Pioglitazone is an oral antidiabetic agent used in the treatment of type 2 diabetes. After administration, pioglitazone decreases insulin resistance in the periphery and liver resulting in increased insulin dependent glucose disposal and decreased hepatic glucose output [1,2]. It is used both as monotherapy and in combination with insulin in the management of type 2 diabetes [3,4]. Pharmacological studies indicate that pioglitazone improves sensitivity to insulin in muscle and adipose tissues and inhibits hepatic gluconeogenesis. Candesartan is a selective angiotensin II type 1 receptor antagonist. The drug finds most significant clinical
use in the treatment of hypertension of all grades [5,6]. Hypertension is frequently accompanied by type 2 diabetes in the same patients and hence, many hypertensive patients are subjected to the combination therapy with an antihypertensive drug and an antidiabetic drug. The combination of pioglitazone and candesartan exerts more beneficial effects on hypertensive cardiovascular injury in hypertension [7].

As per the literature, several LC-MS/MS methods have been reported for the determination of pioglitazone and candesartan individually in biological samples [8–13]. The major disadvantages of all these methods include complicated and expensive extraction procedures or long chromatographic run time. The method proposed by Xue et al. [8] for quantification of pioglitazone in human serum and Levi et al. [9] for quantification of candesartan in human plasma utilizes on-line sample preparation technique, which is expensive equipment involving many stringent method development protocols. Another method reported by Lin et al. [11] for determination of pioglitazone in human plasma is more sensitive but a time-cost sample preparation involving liquid–liquid (L–L) extract, evaporation, drying and reconstitution was used in this method for sample preparation. Some methods [10–12] which can satisfy the quantitation of one drug in biological fluids selectively and sensitively cannot be applied to simultaneous determination pioglitazone and candesartan.

To date, no LC-MS/MS method has been reported for the simultaneous determination of pioglitazone and candesartan in human plasma. For pharmacokinetic and bioequivalence studies of pioglitazone associated with candesartan, it is recommended to perform the quantitation of pioglitazone and candesartan simultaneously. The present work describes a simple, selective and sensitive method, which employs solid-phase extraction technique for sample preparation and liquid chromatography with electrospray ionization-tandem mass spectrometry for simultaneous quantitation of pioglitazone and candesartan in human plasma. The application of this assay method to a clinical pharmacokinetic study in healthy male volunteers following oral administration of pioglitazone and candesartan is described.

2. Experimental

2.1. Materials and reagents

The reference samples of pioglitazone hydrochloride (99.70%) and candesartan (98.06%) were purchased from Neuron Pharma Pvt. Ltd, Goa, India and irbesartan (99.44%) used as an internal standard (IS) in this study, was obtained from Hetero Drugs Ltd, Hyderabad, India. Chemical structures are presented in Fig. 1. Water used for the LC-MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). Acetonitrile and methanol (HPLC grade) were purchased from J.T. Baker (Phillipsburg, USA). Analytical grade formic acid was purchased from Merck Ltd (Mumbai, India). The control human plasma sample was procured from Cauvery Diagnostics and Blood Bank (Secunderabad, India).

2.2. Instrumentation and chromatographic conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a Zorbax SB C18 column (50 × 4.6 mm, 3.5 μm; Agilent Technologies, Santa Clara, CA, USA), a binary LC-20AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU-20 A3) was used for the study. Aliquots of the processed samples (20 μL) were injected into the column, which was kept at ambient temperature. An isocratic mobile phase consisting of a 80:20 (v/v) mixture of acetonitrile and 0.1% formic acid was used to separate the analytes and delivered at a flow rate of 0.8 mL/min into the electrospray ionization chamber of the mass spectrometer. Quantification was achieved with MS–MS detection in positive ion mode for both the analytes and the internal standard using an MDS Sciex API-3000 mass spectrometer (Foster City, CA, USA) equipped with a Turboion-sprayTM interface at 300 °C. The ion spray voltage was set at 5500 V. The source parameters viz. the nebulizer gas, curtain gas and collision gas were set at 4, 12 and 12 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP), focusing potential (FP) and collision cell exit potential (CXP) were 82, 17, 10, 250, 7 V for pioglitazone, 47, 40, 10, 250, 7 V for candesartan and 134.0 for pioglitazone, m/z 357.1 precursor ion to the m/z 134.0 for pioglitazone, m/z 441.3 precursor ion to the m/z 263.1 for candesartan and m/z 429.2 precursor ion to the m/z 207.1 product ion for the IS. Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed by Analyst softwareTM (version 1.4.2). As earlier publications have discussed the details of fragmentation patterns of pioglitazone [13], candesartan [9] and IS [14], we are not presenting the data pertaining to this.

2.3. Preparation of plasma standards and quality controls

Stock solutions of pioglitazone, candesartan and the irbesartan were dissolved in methanol at a concentration of 1 mg/mL.

![Figure 1 Chemical structures of pioglitazone, candesartan and irbesartan (IS).](Image)
From these stock solutions, appropriate dilutions were made to produce working standard solutions using a 50:50 (v/v) mixture of acetonitrile and water as a diluent. Calibration curve (CC) standard solutions of pioglitazone and candesartan in blank plasma were prepared by spiking with an appropriate volume of the working solutions, giving final concentrations of 15, 30, 75, 300, 601, 1202, 1800, 2400 and 3000 ng/mL for pioglitazone, and 5, 10, 30, 60, 120, 240, 360, 487 and 608 ng/mL for candesartan. The CC samples were analyzed along with the quality control (QC) samples for each batch of plasma samples. The QC samples were prepared at five different concentration levels of 15 (LLOQ), 40 (LQC), 401 (MQC-1), 1457 (MQC-2) and 2602 (HQC) ng/mL for pioglitazone and 5 (LLOQ), 15 (LQC), 76 (MQC-1), 303 (MQC-2) and 515 (HQC) ng/mL for candesartan in blank plasma. All the prepared plasma samples were stored at –70 °C.

2.4. Sample processing

A 250 μL aliquot of human plasma sample was mixed with 25 μL of the internal standard working solution (1000 ng/mL of irbesartan). To this, 500 μL of 5% formic acid was added after vortex mixing for 10 s. The sample mixture was loaded onto a Strata-X 33 μm polymeric sorbent cartridge (30 mg/1 mL) that was pre-conditioned with 1.0 mL of methanol followed by 1.0 mL water. The extraction cartridge was washed with 1.0 mL of 5% formic acid followed by 1.0 mL of water. Pioglitazone, candesartan and irbesartan were eluted with 1.0 mL of mobile phase. Aliquot of 20 μL of the extract was injected into the LC-MS/MS system.

2.5. Method validation

The validation of the above method was carried out as per US FDA guidelines [15]. The parameters determined were selectivity, matrix effect, linearity, precision, accuracy, recovery, stability and dilution integrity. Selectivity was assessed by comparing the chromatograms of six different batches of blank plasma obtained from six different sources including one lipemic and one hemolyzed plasma. Sensitivity was determined by analyzing six replicates of plasma samples spiked with the lowest level of the calibration curve concentrations. Matrix effect was checked with six different lots of K$_2$-EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total). For checking the linearity standard calibration curves containing at least nine points (non-zero standards) were plotted (15–3000 ng/mL for pioglitazone and 5–608 ng/mL for candesartan). In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences. Intra-day precision and accuracy were determined by analyzing six replicates at five different QC levels on two different days. Inter-day precision and accuracy were determined by analyzing six replicates at five different QC levels of five different runs. Recoveries of pioglitazone, candesartan and irbesartan were determined by comparing the peak area of extracted analyte standard with the peak area of non-extracted standard. Recoveries of pioglitazone and candesartan were determined at a concentration of 40, 15 (LQC), 1457, 303 (MQC-2) and 2602, 515 (HQC) ng/mL, respectively, whereas for IS recovery was determined at concentration of 1000 ng/mL. Dilution integrity was performed to extend the upper concentration limit with acceptable precision and accuracy. Six replicates each at a concentration of about 1.7 times of the uppermost calibration standard were diluted two- and four-fold with blank plasma. The diluted samples were processed and analyzed. Stability tests were conducted to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. The stock solution stability at room temperature and refrigerated conditions (2–8 °C) was performed by comparing the area response of the analytes (stability samples) with the response of the sample prepared from fresh stock solution. Bench top stability (10 h), processed samples stability (Autosampler stability for 48 h, wet extract stability for 24 h and re-injection stability for 24 h), freeze-thaw stability (three cycles), long-term stability (50 day) were performed at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (≤15% RSD).

2.6. Pharmacokinetic study design

A pharmacokinetic study was performed in healthy male subjects (n=6). The ethics committee approved the protocol and the volunteers provided with informed written consent. Blood samples were collected following oral administration of pioglitazone (30 mg) and candesartan (16 mg) at pre-dose and 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 24 and 36 h, in K$_2$-EDTA vacutainer collection tubes (BD, Franklin, NJ, USA). The tubes were centrifuged at 3200 rpm for 10 min and the plasma was collected. The collected plasma samples were stored at –70 °C till their use. Plasma samples were spiked with the IS and processed as per the extraction procedure described earlier. Along with the clinical samples, the QC samples at low, middle 1, middle 2 and high concentration levels were also assayed in triplicate. Plasma concentration-time profile of candesartan was analyzed by non-compartmental method using WinNonlin Version 5.1.

3. Results and discussion

3.1. Method development

Mass parameters were tuned in both positive and negative ionization modes for the analytes. Good response was found in positive ionization mode. Data in the MRM mode were considered, which showed better selectivity. Chromatographic conditions, especially the composition of the mobile phase, were optimized through several trials to achieve good resolution and increased intensity of the signals of the analytes, as well as short run time. The presence of a small amount of formic acid in the mobile phase improved the detection of the analytes. It was found that a mixture of acetonitrile and 0.1% formic acid (80:20, v/v) could achieve this purpose and was finally adopted as the mobile phase. Zorbax SB C$_{18}$ (50×4.6 mm, 3.5 μm) column gave good peak shapes and response even at lowest concentration level for both the analytes and IS. The mobile phase was operated at a flow rate of 0.8 mL/min. The retention time of pioglitazone, candesartan and the IS was low enough (0.7, 1.6 and 0.9 min) allowing a small run time of 2.7 min. A simple
solid-phase extraction (SPE) technique was employed for the sample preparation in this work and provided high recoveries of the drugs. At the initial stages of this work, several compounds were tried for finding out a suitable IS in this analysis and finally irbesartan was found to be the best for the purpose.

3.2. **Selectivity and chromatography**

The degree of interference by endogenous plasma constituents with the analytes and the IS was assessed by inspection of chromatograms derived from processed blank plasma sample. As shown in Figs. 2 and 3, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free plasma at the retention time of the analytes.

3.3. **Sensitivity**

The lowest limit of reliable quantification for the analytes was set at the concentration of the LLOQ. The precision and accuracy at LLOQ concentration were found to be 2.51% and 99.22% for pioglitazone, 2.13% and 96.85% for candesartan.

3.4. **Matrix effect**

No significant matrix effect was observed in all the six batches of human plasma for the analytes at low and high quality control concentrations. The precision and accuracy for pioglitazone at LQC concentration were found to be 1.33% and 102.68%, and at HQC level they were 1.08% and 99.69%, respectively. Similarly, the precision and accuracy for candesartan at LQC concentration were found to be 2.81% and 97.64%, and at HQC level they were 1.98% and 98.87%, respectively.

3.5. **Linearity**

The nine-point calibration curve was found to be linear over the concentration range of 15–3000 ng/mL for pioglitazone and 5–608 ng/mL for candesartan. After comparing the two weighting models (1/x and 1/x^2), a regression equation with a weighting factor of 1/x^2 of the drug to the IS concentration was found to produce the best fit for the concentration–detector response relationship for both the analytes in human plasma. The mean correlation coefficient of the weighted calibration curves generated during the validation was 0.99.

![Typical MRM chromatograms of pioglitazone (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C).](image-url)

**Figure 2** Typical MRM chromatograms of pioglitazone (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C).
Figure 3  Typical MRM chromatograms of candesartan (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C).

Table 1  Precision and accuracy data for pioglitazone and candesartan in human plasma samples.

| Analyte | Concentration added (ng/mL) | Intra-day precision and accuracy | Inter-day precision and accuracy |
|---------|-----------------------------|---------------------------------|---------------------------------|
|         |                             | Concentration found (mean; ng/mL) | Precision (%) | Accuracy (%) | Concentration found (mean; ng/mL) | Precision (%) | Accuracy (%) |
| Pioglitazone |                            |                                    |                    |                |                                    |                    |                |
| 15.1    | 14.3                        | 4.8                               | 94.3              | 14.3           | 5.2                               | 94.8              |
| 40.1    | 39.0                        | 2.9                               | 97.3              | 38.6           | 3.6                               | 96.4              |
| 400.7   | 372.0                       | 4.1                               | 92.9              | 381.3          | 5.8                               | 95.2              |
| 1457.0  | 1400.8                      | 2.5                               | 96.1              | 1373.7         | 2.8                               | 94.3              |
| 2601.8  | 2507.2                      | 3.0                               | 96.4              | 2460.9         | 2.9                               | 94.6              |
| Candesartan |                            |                                    |                    |                |                                    |                    |                |
| 5.1     | 5.2                         | 4.0                               | 102.3             | 5.0            | 6.2                               | 98.6              |
| 15.2    | 15.8                        | 2.6                               | 104.4             | 15.5           | 3.8                               | 102.3             |
| 75.8    | 79.9                        | 2.5                               | 105.4             | 79.0           | 3.3                               | 104.1             |
| 303.3   | 320.0                       | 20.0                              | 105.5             | 316.2          | 2.7                               | 104.3             |
| 514.9   | 521.5                       | 2.3                               | 101.3             | 514.5          | 3.1                               | 99.9              |
3.6. Precision and accuracy

As shown in Table 1, the precision and accuracy of each analyte in the intra- and inter-day runs were within ±15% at LQC, MQC-1, MQC-2 and HQC concentrations and within ±20% at LLOQ QCs.

3.7. Extraction efficiency

Six replicates at low, medium and high quality control concentration for pioglitazone and candesartan were prepared for recovery determination. The recoveries of analytes and IS were good and reproducible. The mean overall recoveries (with the precision range) of pioglitazone, candesartan and IS were 98.15 ± 1.73% (1.89–5.71%), 77.66 ± 3.15% (1.33–3.15%) and 75.53 ± 0.85% (1.19–2.78%), respectively.

3.8. Dilution integrity

The upper concentration limits can be extended to 4800 ng/mL for pioglitazone and 975 ng/mL for candesartan by 1/2 and 1/4 dilutions with screened human blank plasma. The mean back calculated concentrations for 1/2 and 1/4 dilution samples were within 85–115% of their nominal value. The coefficients of variation (%CV) for 1/2 and 1/4 dilution samples were less than 10%.

3.9. Stability studies

In the different stability experiments carried out viz. bench top stability (10 h), autosampler stability (48 h), repeated freeze-thaw cycles (three cycles), reinjection stability (24 h), wet extract stability (24 h at 2–8°C) and long-term stability at −70°C for 50 day the mean % nominal values of the analytes

| Stability test | Pioglitazone | | Candesartan | |
|----------------|-------------|-----------------|-------------|
| QC (spiked concentration, ng/mL) | Mean±SD | Accuracy/stability (%) | Precision (%) | QC (spiked concentration, ng/mL) | Mean±SD | Accuracy/stability (%) | Precision (%) |
| Autosampler stability (at 10°C for 48 h) | 40.1 | 40.0±1.6 | 102.4 | 3.8 | 15.2 | 15.5±0.3 | 102.5 | 2.2 |
| Wet extract stability (at 2–8°C for 24 h) | 40.1 | 40.6±0.5 | 101.3 | 1.1 | 15.2 | 15.5±0.3 | 102.5 | 2.1 |
| Bench top stability (10 h at room temperature) | 40.1 | 39.4±0.6 | 98.4 | 1.4 | 15.2 | 15.0±0.3 | 99.0 | 2.3 |
| Freeze-thaw stability (three cycles) | 40.1 | 40.8±0.5 | 101.9 | 1.3 | 15.2 | 15.6±0.9 | 102.7 | 5.6 |
| Reininjection stability (24 h) | 40.1 | 37.9±3.0 | 94.5 | 7.9 | 15.2 | 16.2±0.3 | 106.7 | 1.6 |
| Long-term stability (at −70°C for 50 day) | 40.1 | 40.4±0.6 | 100.8 | 1.5 | 15.2 | 14.8±0.2 | 97.9 | 1.1 |

Figure 4 Mean plasma concentration-time profile of pioglitazone (A), candesartan (B), in human plasma following oral dosing of pioglitazone (30 mg) and candesartan (16 mg) tablet to healthy volunteers.
were found to be within ±15% of the predicted concentrations for the analytes at their LQC and HQC levels (Table 2). Thus, the results were found to be within the acceptable limits during the entire validation.

3.10. Pharmacokinetic study results

In order to verify the sensitivity and selectivity of this method in a real-time situation, the present method was used to test for pioglitazone and candesartan concentrations in human plasma samples collected from healthy male volunteers (n=6). The mean plasma concentrations vs time profiles of pioglitazone and candesartan are shown in Fig. 4. The pharmacokinetic parameters estimated are shown in Table 3. These values were in close proximity when compared with earlier reported values [16,17].

4. Conclusions

The LC-MS/MS assay method described in this paper is rapid, simple, specific and sensitive for quantification of pioglitazone and candesartan in human plasma and is fully validated as per the FDA guidelines. To the best of our knowledge, this is the first report on simultaneous assay of pioglitazone and candesartan in any of the matrix without compromising on the reported sensitivity for each analyte. The method was found to be suitable for pharmacokinetic studies in humans. The simple solid-phase extraction method gave consistent and reproducible recoveries for the analytes from plasma. The method provided good linearity. A sample turnover rate of less than 2.7 min makes it an attractive procedure in high-throughput bioanalysis of pioglitazone and candesartan.

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References

[1] C.A. Hofmann, C.W. Edwards, J.R. Colca, et al., Treatment of insulin-resistant mice with the oral antidiabetic agent pioglitazone: evaluation of liver GLUT2 and phosphoenolpyruvate carboxykinase expression, Endocrinology 130 (1) (1992) 735–740.

[2] R.F. Kletzien, L.A. Foellmi, P.K. Harris, et al., Adipocyte fatty acid-binding protein: regulation of gene expression in vivo and in vitro by an insulin-sensitizing agent, Mol. Pharmacol. 42 (4) (1992) 558–562.

[3] H.Y. Zhang, S.R. Reddy, T.A. Kotchen, Antihypertensive effect of pioglitazone is not invariably associated with increased insulin sensitivity, Hypertension 24 (1994) 106–110.

[4] J. Chilcott, P. Tappenden, M. Lloyd, et al., A systematic review of the clinical effectiveness of pioglitazone in the treatment of type 2 diabetes mellitus, Clin. Ther. 23 (11) (2001) 1792–1823.

[5] A.D. Dobrian, S.D. Schriver, A.A. Khrabi, et al., Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity, Hypertension 43 (2004) 48–56.

[6] C.H. Gleiter, C. Jagle, U. Gresser, et al., Candesartan, Cardiovasc. Drug Rev. 22 (4) (2004) 263–284.

[7] T. Nakamura, E. Yamamoto, K. Kataoka, et al., Beneficial effects of pioglitazone on hypertensive cardiovascular injury are enhanced by combination with candesartan, Hypertension 51 (2008) 296–301.

[8] Y.J. Xue, K.C. Turner, J. Pursley, et al., 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 (2003) 215–226.

[9] M. Levi, G. Wuerzner, E. Ezan, et al., Direct analysis of valsartan or candesartan in human plasma and urines by on-line solid phase extraction coupled to electrospray tandem mass spectrometry, J. Chromatogr. B 877 (2009) 919–926.

[10] C. Hess, F. Musshoff, B. Madea, Simultaneous identification and validated quantification of 11 oral hypoglycaemic drugs in plasma by electrospray ionisation liquid chromatography–mass spectrometry, Anal. Bioanal. Chem. 400 (2011) 33–41.

[11] J.Z. Lin, W. Ji, D. Desai-Krieger, et al., Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-/MS/MS, J. Pharm. Biomed. Anal. 33 (2003) 101–108.

[12] S.T. Prajapati, P.K. Patel, M. Patel, et al., Development and validation of the liquid chromatography–tandem mass spectrometry method for the quantitative estimation of candesartan and its pharmacokinetic application, Biomed. Chromatogr. 24 (2010) 1342–1349.

[13] S.Y.S.G.B. Prasad, S. Shiva Kumar, T. Sudhir, et al., LC/MS/MS method for the simultaneous estimation of losartan potassium and irbesartan in rat plasma, Int. J. Pharm. Pharm. Sci. 1 (2009) 206–215.

[14] US DHHS, FDA, CDER. Guidance for Industry: Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CV), 2001. Available at: http://www/fda.gov/cder/guidance/index.htm.

[15] P. Sripalakit, P. Neamhom, A. Saraphanchotiwitthaya, High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study, J. Chromatogr. B 843 (2006) 164–169.

[16] E.A. Junior, L.F. Duarte, L. Oliveira, et al., Evaluation of the pharmacokinetic interaction between candesartan cilexetil and felodipine, J. Bioequiv. Availab. 3 (2011) 5–10.