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Development of a high-performance liquid chromatography method for simultaneous determination of pioglitazone and felodipine in pig serum: application to pharmacokinetic study

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ABSTRACT: A simple and sensitive high-performance liquid chromatographic method was developed and validated for simultaneous estimation of pioglitazone and felodipine in pig serum. The present method consists of protein precipitation, extraction of analytes from pig serum into dichloromethane and separation using reversed-phase C_{18} column. Nitrendipine was used as an internal standard and the eluent was monitored by UV detector at 240 nm. The mobile phase used was acetonitrile and 50 mM ammonium acetate buffer at a flow rate of 1 mL/min. The retention times for pioglitazone, felodipine and nitrendipine were found to be 5.12, 10.53 and 7.14 min, respectively. The intraday and inter-day coefficient of variation and percent error values of assay method were less than 7% and mean recovery was more than 94% for each analyte, and the method was found to be precise, accurate and specific during study. The method was successfully applied for pharmacokinetic study of pioglitazone and felodipine from bioadhesive buccal tablet after buccal administration to pigs. The C_{Max}, T_{Max}, and AUC_{0-24} of pioglitazone and felodipine from buccal tablet were found to be 394.6 ng/mL, 5.6 h, 2624.2 ng h/mL and 44.4 ng/mL, 5.5 h, 275.8 ng h/mL, respectively. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: pioglitazone; felodipine; reversed-phase high-performance liquid chromatography; pig serum; pharmacokinetics

Introduction

Pioglitazone hydrochloride (PIO), 5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-thiazolidinedione monohydrochloride (Fig. 1a) is an antihyperglycemic agent that acts primarily by reducing insulin resistance. It is used in the management of type 2 diabetes (non-insulin-dependent diabetes mellitus; Fahim *et al*., 2009). PIO acts by improving sensitivity to insulin in muscles and adipose tissue, and glycemic control, while reducing circulating insulin levels. PIO is rapidly absorbed after oral administration and is extensively metabolized by hydroxylation and oxidation in the liver (bioavailability is about 80%; Eckland and Danhof, 2000). Felodipine (FDP), 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylic acid ethyl methyl ester (Fig. 1b), a calcium channel blocker belonging to dihydropyridines, is used as a potent peripheral vasodilator, which effectively reduces blood pressure when given at doses of 5–20 mg per day. After a single, 20 mg oral dose of FDP, peak plasma concentrations are achieved within 2.5–5 h (Edgar *et al*., 1987). It was reported to be well absorbed following oral administration, but undergoes extensive first pass metabolism; leading to poor bioavailability (Karavas *et al*., 2005). Patients with hyperglycemia also suffer from hypertension; therefore, a combination of two drugs is prescribed to patients. Currently there is no combined dosage form available and it is available as individual tablets of FDP (Plendil) and PIO (Actos) (*Physician's Desk Reference*, 2000). Since PIO and FDP suffer from first-pass hepatic metabolism, an alternative mode of delivery system like buccal delivery system is desirable to improve the bioavailability.

For pharmacokinetic studies, a method that allows an accurate measurement of low concentrations of PIO and FDP in serum is needed. Literature survey reveals that several methods have been used for quantification of PIO using high-performance liquid chromatography (HPLC) in tablet formulation (Lotfy Saber, 2008); in human serum and biological samples using HPLC with tandem mass spectrometry (LC/MS/MS; Xue *et al*. 2003), potentiometric sensors (Mostafa and Al-Majed, 2008) and micellar electrokinetic chromatography (Radhakrishna, *et al*., 2002). The existing analytical methods for FDP include in human plasma by capillary gas chromatography (Ahnoff, 1984), HPLC coupled to tandem mass spectrometry (Migliorança *et al*., 2005),

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Abbreviations used: FDP, felodipine; PIO, pioglitazone hydrochloride.

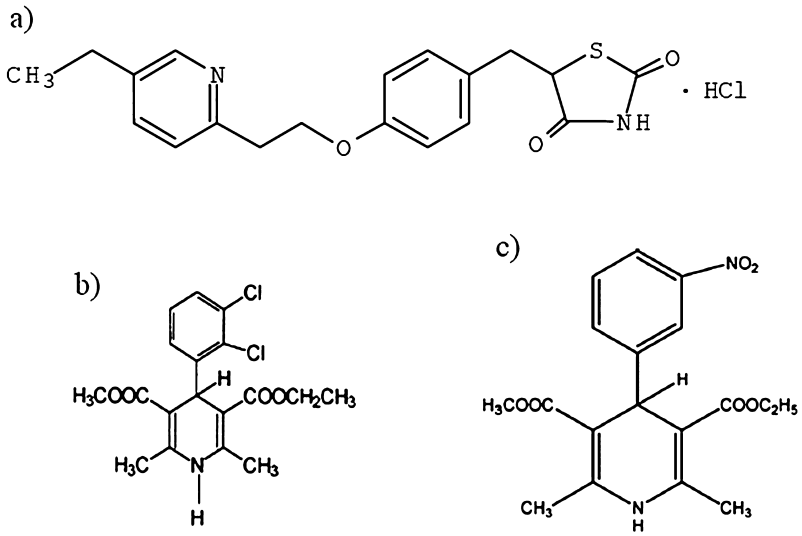

Figure 1. Structure of (a) pioglitazone hydrochloride, (b) felodipine and (c) nitrendipine.

high-selectivity gas chromatography (Ahnoff *et al*., 1987) and HPLC (Soons *et al*., 1990; Lindmark *et al*., 2002; Lopez *et al*., 2000); and in tissue samples by column-switching liquid chromatography–tandem mass spectrometry (Henig and Bucheli, 2002). The drug metabolites in plasma have been determined by HPLC (Gabrielsson, *et al*., 1992); gas chromatography (GC; Dru *et al*., 1995) and capillary GC (Nishioka *et al*., 1991). The reported methods so far available are meant for quantification of single analyte only; no method is available for simultaneous estimation. The disadvantages of the reported methods include cost, time-consuming sample clean-up, laborious extraction steps, derivatization, tedious sample preparation, use of more than one column and long chromatographic runs and low sensitivity, which are not suitable in all conditions. Therefore a sensitive method is required to monitor the plasma concentrations of PIO and FDP and estimate the pharmacokinetics.

The objective of the present work was to develop a sensitive HPLC method for simultaneous determination of PIO and FDP in pig serum. Both the drugs have high first-pass metabolism and design of a buccal dosage form may be advantageous. For the evaluation of new drug delivery systems like buccal drug delivery, animal studies are desired during preclinical evaluation, before conducting clinical experiments in humans. Therefore in the present investigation pig was selected as an animal model because it has been the reported animal for the evaluation of other buccal drug delivery systems (Vamshi *et al*., 2007) and there is a close resemblance in the anatomical structure of the buccal membrane between pig and human (Lesh *et al*., 1989). The advantages of the present method include the simple, single-step extraction procedure using inexpensive chemicals, and short run time. The present method was also successfully applied for the study of pharmacokinetics of FDP and PIO from bioadhesive buccal tablets in pigs.

Experimental

Materials

Pioglitazone hydrochloride (purity, 99.6%), felodipine (purity, 99.3%) and nitrendipine (purity, 99.7%) pure samples were gifted respectively by Dr Reddy's Laboratories, Hyderabad, India, Orchid, Chennai, India and US Vitamins, Mumbai, India. Acetonitrile, methanol (HPLC), ammonium acetate and sodium hydroxide (GR) were purchased from Merck, Mumbai, India. Double-distilled water was used during the entire HPLC procedure.

Chromatographic conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AT solvent module, SPD10A UV–vis detector with LC10 software. The analytical column used was a C_{18} column (Inertsil, GL Sciences Inc., Japan) with a length of 25 cm, 4.6 mm i.d., particle size 5 µm) and the chromatography was carried at a temperature of 40°C. The mobile phase consisted of acetonitrile and 50 mM ammonium acetate buffer (pH was adjusted to 5 with glacial acetic acid) at a ratio of 67:33 v/v. The elute was monitored at 240 nm, at a flow rate of 1 mL/min. The injection volume was 20 µL and detector sensitivity was set to 0.0005 AUFS.

Preparation of calibration standards and quality control samples

The stock solutions of PIO, FDP and NTDP were prepared in methanol at a concentration of 1 mg/mL each. NTDP (Fig. 1c) was used as an internal standard (IS). The working solutions of 10 µg/mL for PIO and FDP and 1.5 µg/mL for NTDP were prepared by appropriately diluting the respective stock solutions. PIO and FDP working solutions were used to prepare the spiking stock solutions for construction of respective calibration curves. PIO at a concentration of 1, 5, 10, 25, 50, 100, 250, 500, 1000, 2500 and 5000 ng/mL and FDP 1, 5, 10, 25, 50, 100, 250, 500, 1000, 2000 and 4000 ng/mL were used to prepare an 11-point calibration curve. Quality control (QC) samples at three different levels for PIO (2.5, 2250 and 4500 ng/mL) and FDP (2.5, 1750 and 3500 ng/mL) were prepared. All the stock solutions were refrigerated (4°C) when not in use. Calibration standards and QC samples were prepared in bulk by spiking 100 µL of respective spiking stock solutions to 1 mL of control pig serum and were stored at −20°C until analysis.

Sample preparation for analysis

An aliquot (1.2 mL) of pig serum containing PIO and FDP was transferred into screw capped tubes and 100 µL of IS (1500 ng/mL of NTDP) was added and vortexed for 2 min. Sodium hydroxide solution (1 M), 0.3 mL was added and vortexed for 3 min followed by the addition of 8 mL of dichloromethane. This was vortexed for 5 min and centrifuged (MIKRO 220R Hettich, Germany) at 5000 rpm for 15 min. The organic layer was

separated and allowed to evaporate in a vacuum oven (Sheldon Manufacturing Inc., Cornelius, USA). The evaporated residue was reconstituted with 100 µL of methanol and 20 µL of the reconstituted sample was injected into HPLC system.

Assay validation

The intraday and inter-day precision and accuracy of the assay was determined by percentage coefficient of variation (CV) and percentage relative error (RE) values, respectively, based on reported guidelines (FDA, 2001). Samples containing 2.5, 2250 and 4500 ng/mL for PIO and 2.5, 1750 and 3500 ng/mL for FDP were spiked for the determination of precision and accuracy. Five replicates at each concentration were processed as described in the previous section on days 1, 3, 5 and 10 to determine intra-day and inter-day precision and accuracy. The percentage CV and percentage RE values were calculated using the following equations:

$$\%CV = (SD/mean) \times 100$$

$$\%RE = [(measured\ value - theoretical\ value)/theoretical\ value] \times 100$$

Lower limit of quantitation and limit of detection

The lower limit of quantitation (LLOQ) is defined as the lowest concentration of analyte that can be determined with acceptable precision and accuracy. The limit of detection (LOD) is a parameter that provides the lowest concentration in a sample that can be detected from background noise but not quantitated. LOD was determined using signal-to-noise ratio (S/N) of 3:1 by comparing test results from samples with known concentrations of analytes with blank samples.

Recovery

The recovery of PIO and FDP was determined for QC samples at concentration of 2.5, 2250 and 4500 ng/mL and 2.5, 1750 and 3500 ng/mL, respectively. Five replicates of each QC sample were extracted and injected into the HPLC system. The extraction recovery at each concentration was calculated using following equation:

$$Recovery = (peak\ area\ after\ extraction/peak\ area\ after\ direct\ injection) \times 100$$

Stability studies

To ensure the reliability of results in handling and storing of serum samples and stock solutions, stability studies were carried out at three concentration levels for PIO and FDP at 2.5, 2250 and 4500 ng/mL and 2.5, 1750 and 3500 ng/mL, respectively. The stability of spiked pig serum stored at room temperature (bench-top stability) was evaluated for 12 h. Freeze and thaw stability was performed over three freeze–thaw cycles by thawing at room temperature for 2–8 h and then refreezing at −20°C for 12–24 h. The long-term stability of PIO and FDP in pig serum was assessed by carrying out the experiment after 30 days of storage at −20°C. The stock solution stability of PIO, FDP (1500 ng/mL for each) and NTDP (1500 ng/mL) were determined at room temperature for 12 h and upon refrigeration (4°C) for 14 days. The concentration of PIO and FDP after each storage period was related to the initial concentration as determined for the samples that were freshly prepared.

Robustness

To determine the robustness of method, the final experimental conditions were altered and the results were examined. The flow rate was varied by 1 ± 0.2 mL/min. The percentage of organic strength was varied by 67 ± 2%. Buffer concentration was varied by 50 ± 10 mM, pH varied by 5.0 ± 0.2 units and column temperature was varied by 40 ± 5°C.

Application to pharmacokinetic study

The pharmacokinetic study was conducted in six pigs (body weight 30 ± 5 kg), with permission from the institutional animal ethical committee, University College of Pharmaceutical Sciences, Warangal, India. Bioadhesive buccal tablet containing 15 mg of PIO and 5 mg of FDP were administered by buccal route to the pigs and blood samples (5 mL) were collected at intervals of 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. All blood samples were allowed to clot and centrifuged for 10 min at 5000 rpm. The serum was separated and transferred into clean micro centrifuge tubes and stored at −20°C until HPLC analysis. Pharmacokinetic parameters, peak serum concentration (C_{Max}), time to reach peak concentration (T_{Max}) and area under the curve (AUC) for PIO and FDP were obtained for each pig using the computer program Kinetica 2000 (Version 3.0, Innaphase Corporation, Philadelphia, USA) meant for calculation of model independent parameters.

Results and discussion

Chromatography

The chromatographic conditions and sample preparation for the proposed method were optimized to suit the preclinical pharmacokinetic studies. Figure 2 shows typical chromatograms of pig blank serum (PIO and FDP free), serum spiked with PIO and FDP, and pig serum after buccal administration of 15 and 5 mg of PIO and FDP in combined buccal dosage form. The retention times of PIO, FDP and NTDP, respectively, were 5.12, 10.53 and 7.14 min with a total run time of 13 min. The analytical processes of PIO, FDP and IS were resolved with good symmetry. No endogenous interfering peaks were observed in individual pig blank serum at the retention times of PIO, FDP and IS, thereby confirming the specificity of the analytical method. System suitability parameters for the method were as follows: theoretical plates for PIO, FDP and IS were 1672, 2458 and 1790, respectively. Tailing factors were 1.1, 1.3 and 1.2, respectively, for PIO, FDP and IS. The resolution between PIO and IS was 3.6 and the resolution between IS and FDP was found to be 5.3.

Quantification and calibration curve

The ratios of peak area of PIO to IS and FDP to IS were used respectively for the quantification of PIO and FDP in pig serum. The calibration curves of PIO and FDP were constructed over a period of 10 days, each calibration curve originating from a new set of extractions. Calibration curves were linear in the concentration range of 1–5000 ng/mL for PIO and 1–4000 ng/mL for FDP. The regression equation was $y = mx + c$, where y represents the peak area ratio of PIO to IS/FDP to IS, x represents the concentrations of PIO/FDP, m is slope of the curve and c is the intercept. The equation of calibration curve of PIO and FDP obtained from 11 points, respectively, were $y = 0.0002x + 0.0041$ ($r^2 = 0.998$) and $y = 0.0034x + 0.3414$ ($r^2 = 0.993$).

Accuracy and precision

The accuracy and precision of the method were evaluated for two analytes with QC samples at concentrations of 2.5, 2250 and 4500 ng/mL for PIO and 2.5, 1750 and 3500 ng/mL for FDP respectively. The inter-day accuracy and precision were determined on four different days and the results are shown in Table 1. The inter-day and intra-day precisions of the QC samples for both the drugs were satisfactory with CV less than 7%. The %RE for both dugs was found to be less than 4.

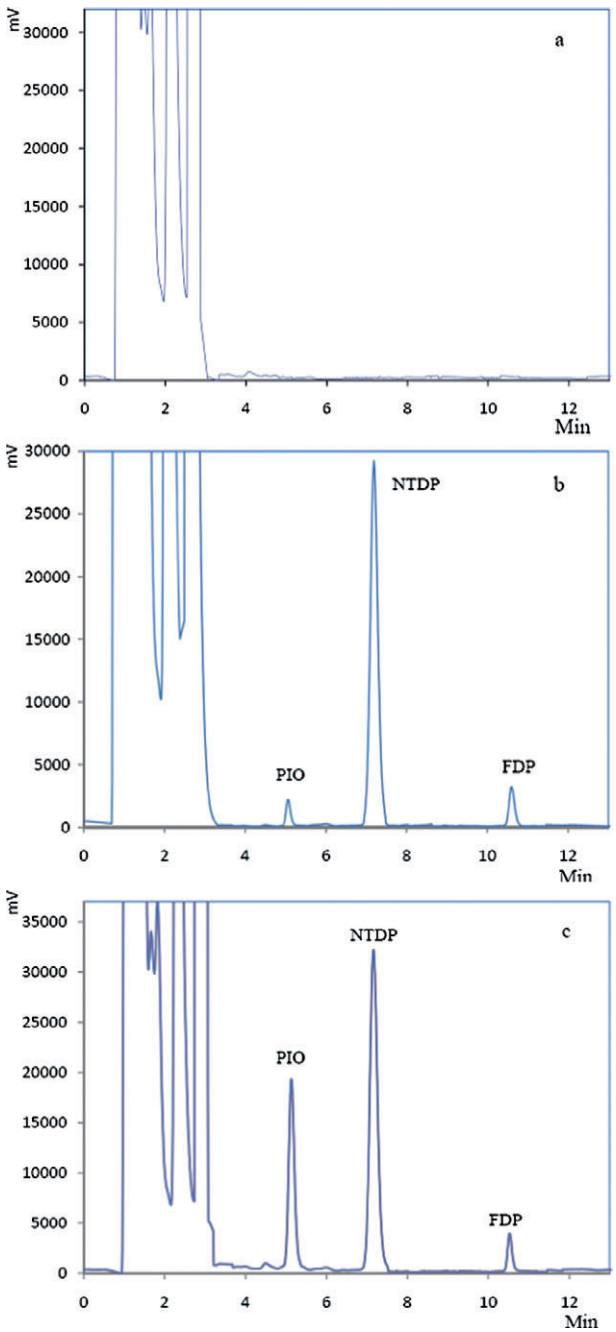

Figure 2. HPLC chromatograms of (a) blank pig serum, (b) pig serum spiked with 2.5 ng of PIO, 2.5 ng of FDP and 150 ng of NTDP and (c) pig serum collected at 12 h, after administration of buccal tablet. Corresponding concentrations of PIO and FDP were 35.7 and 2.4 ng/mL. The retention times of PIO, FDP and NTDP, respectively, were 5.12, 10.53 and 7.14 min.

LLOQ and LOD

LLOQ was established by determining the concentrations of four spiked calibration standards. The LLOQ of the method was found to be 1 ng/mL for PIO with CV less than 11% and an accuracy of 93–105%. The LLOQ of the method was found to be 1 ng/mL for FDP with CV less than 13% and an accuracy of 89–109%. The LOD was determined to be 0.75 ng/mL for both PIO and FDP based on a S/N ratio of 3:1.

Recovery

The extraction recovery was determined by standard addition at three different concentrations, 2.5, 2250, 4500 ng/mL for PIO and 2.5, 1750, 3500 ng/mL for FDP, and one concentration (150 ng/mL) for IS. The extraction recovery was calculated by comparing the peak areas of the prepared standard samples with those of standard solutions; the results are shown in Table 2. The extraction recovery of PIO at 2.5, 2250 and 4500 ng/mL was 98.4, 98.2 and 97.6%, respectively, and the recovery of FDP at 2.5, 1750 and 3500 ng/mL was 96.2, 94.3 and 96.7%, respectively. The mean recovery of NTDP was found to be 96.4%. The recovery of PIO and FDP using the described procedure was consistent and efficient.

Stability

The stability of stock solutions was performed at 1500 ng/mL for PIO and FDP. After storage for 14 days at 4°C and at room temperature for 12 h, more than 99% of PIO and FDP remained unchanged, based on peak areas in comparison with freshly prepared solution. The results suggest that PIO and FDP in stock solutions were stable for at least 14 days when stored at 4°C and for 12 h at room temperature. Bench-top stability of PIO and FDP in serum was investigated and the results revealed that PIO and FDP in serum were stable with average percentages of 97, 98, 100% and 99, 99, 98% respectively. The repeated freezing and thawing for three cycles of serum samples spiked with PIO and FDP showed a mean percentage concentration of 95, 99, 100% and 99, 99, 98%, respectively. Long-term stability of the PIO and FDP in serum at −20°C showed mean percentage concentration of 96, 99, 99% and 97, 99, 99%, respectively. The results (Table 3) of stability study indicated that PIO and FDP were stable in the studied conditions.

Robustness

The results of robustness study are shown in Table 4. It can be seen that in every employed condition, the chromatographic parameters are in accordance with established value (Lopez, *et al*., 2000). In all the employed conditions, the tailing factor for PIO, FDP and NTDP was found to be less than 1.4 and all analytes were well separated under the changes carried out. The resolution ranged between PIO and IS was 2.8–4.6 and the resolution between IS and FDP was 4.0–6.5. Considering the result of modifications in the system suitability parameters and the specificity of the method, it can be concluded that the method conditions are robust.

Application to pharmacokinetic study

The method was applied to the analysis of serum samples obtained after buccal administration of a mucoadhesive buccal tablet containing 15 mg of PIO and 5 mg of FDP in pigs. Figure 3(a, b) depicts the mean serum profiles of PIO and FDP after administration of the buccal tablet. The pharmacokinetic parameters estimated are shown in Table 5. The C_{Max}, T_{Max}, AUC_{0-24} and AUC_{Total} for PIO and FDP after administration of buccal tablet were found to be, respectively, 394.6 ng/mL, 5.6 h and 2624.2 ng h/mL; and 44.4 ng/mL, 5.5 h and 275.8 ng h/mL.

Conclusions

A simple, sensitive and reliable method for the determination of pioglitazone and felodipine over the concentration ranges

Table 1. Intra-day and inter-day precision and accuracy data for assay of PIO and FDP in pig serum ($n = 5$)

Added concentration (ng/mL)	Calculated concentration (ng/mL)		%CV		% Relative error	
	Intraday	Inter-day	Intraday	Inter-day	Intraday	Inter-day
Pioglitazone						
2.5	2.5	2.5	2.1	3.0	2.4	−1.5
2250	2213.2	2235.4	6.1	3.4	−3.5	−1.8
4500	4503.2	4480.1	5.4	3.3	0.6	−2.4
Felodipine						
2.5	2.5	2.4	4.0	4.4	0.4	−3.2
1750	1747.4	1735.2	0.7	0.8	−0.1	−0.8
3500	3510.2	3461.2	2.3	3.4	0.3	−1.1

Table 2. Recovery and accuracy of the proposed method

Concentration (ng/mL)	Absolute recovery				Accuracy (%)		
	Concentration (ng/mL) (mean ± SD)	Mean (%) ± SD ($n = 5$)	Range (min–max)	%CV	Mean ± SD ($n = 5$)	Range (min–max)	%CV
Pioglitazone							
2.5	2.5 ± 0.1	98.4 ± 2.9	94.2–99.8	2.8	95.9 ± 3.2	91.6–98.8	3.4
2250	2215.4 ± 53.8	98.2 ± 2.5	91.4–98.7	2.6	98.1 ± 1.7	95.5–99.9	1.7
4500	4395.5 ± 140.6	97.6 ± 3.2	93.8–102.4	3.3	96.1 ± 3.7	90.2–99.8	3.8
Felodipine							
2.5	2.4 ± 0.1	96.2 ± 4.2	89.6–97.8	4.2	96.3 ± 3.7	91.6–100.8	3.9
1750	1650.8 ± 44.5	94.3 ± 2.5	90.4–96.8	2.7	97.6 ± 2.4	94.0–100.4	2.5
3500	3385.5 ± 115.8	96.7 ± 3.3	92.8–99.1	3.4	97.1 ± 2.6	95.0–101.2	2.7

Table 3. Stability study results of the proposed method

Stability	Spiked concentration (ng/mL)	Calculated comparison sample concentration (ng/mL)[d]		Calculated stability sample concentration (ng/mL)[d]		Avg%
		Mean ± SD	% CV	Mean ± SD	% CV	
Pioglitazone						
Bench-top[a]	2.5	2.5 ± 0.1	4.8	2.4 ± 0.2	9.1	97
	2250	2195.4 ± 63.5	2.8	2155.2 ± 78.5	3.6	98
	4500	4410.6 ± 98.4	2.2	4415.2 ± 82.8	1.9	100
Freeze and thaw[b]	2.5	2.4 ± 0.2	6.5	2.3 ± 0.2	9.9	95
	2250	2208.2 ± 86.3	3.9	2196.7 ± 93.1	4.2	99
	4500	4503.7 ± 104.4	2.3	4494.4 ± 105.2	2.3	100
Long-term[c]	2.5	2.5 ± 0.1	4.8	2.4 ± 0.2	6.3	96
	2250	2205.4 ± 83.3	3.7	2195.2 ± 88.2	4.0	99
	4500	4480.6 ± 108.4	2.4	4466.4 ± 107.8	2.4	99
Felodipine						
Bench-top[a]	2.5	2.4 ± 0.2	8.7	2.4 ± 0.3	12.5	99
	1750	1725.4 ± 51.5	3.0	1710.3 ± 59.5	3.5	99
	3500	3465.1 ± 68.2	1.9	3408.5 ± 82.1	2.4	98
Freeze and thaw[b]	2.5	2.4 ± 0.1	5.0	2.38 ± 0.2	8.0	99
	1750	1730.5 ± 47.2	2.7	1715.3 ± 58.7	3.4	99
	3500	3490.2 ± 52.6	1.5	3428.5 ± 81.4	2.4	98
Long-term[c]	2.5	2.4 ± 0.2	8.7	2.3 ± 0.2	8.5	97
	1750	1725.4 ± 51.5	3.0	1703.3 ± 80.6	4.7	99
	3500	3465.1 ± 68.2	2.0	3425.4 ± 95.7	2.8	99

[a] After 12 h at room temperature; [b] after three freeze–thaw cycles; [c] after 30 days at −20°C; [d] values are mean ± SD ($n = 3$).

Table 4. Robustness data of the developed HPLC method

Parameter	Modification	Retention time (min)			Tailing factor			Theoretical plates			Resolution	
		PIO	NTDP	FDP	PIO	NTDP	FDP	PIO	NTDP	FDP	PIO and NTDP	NTDP and FDP
Mobile phase ratio (v/v), acetonitrile : buffer (pH 5.0)	65:35	5.16	7.19	10.81	1.13	1.06	1.35	1642	1783	2589	3.7	5.6
	67:33	5.14	7.16	10.54	1.00	1.00	1.22	1629	1779	2466	3.7	5.2
	69:31	4.63	6.15	8.77	0.98	0.96	1.18	1311	1312	1707	2.8	4.0
Flow rate (mL/min)	0.8	6.42	8.92	13.14	1.12	1.25	1.25	2526	2760	3833	4.6	6.5
	1.0	5.14	7.16	10.54	1.02	1.14	1.05	916	1779	2466	3.7	5.2
	1.2	4.32	6.02	8.88	1.00	0.98	1.00	2589	2235	2735	3.4	5.2
Buffer concentration (mM)	40	5.16	7.15	10.51	1.35	1.18	1.06	916	1773	2452	3.7	5.2
	50	5.14	7.16	10.54	1.18	0.94	1.16	1629	1779	2466	3.7	5.2
	60	4.96	7.17	10.52	1.24	0.92	1.12	1517	1783	2457	4.0	5.2
pH	4.8	5.16	7.05	10.36	1.08	1.28	1.27	1642	1724	2383	3.4	5.1
	5.0	5.14	7.16	10.54	0.96	1.24	1.15	916	1779	2466	3.7	5.2
	5.2	5.04	7.14	10.48	1.00	1.12	1.20	1566	1768	2438	3.8	5.1
Temperature (°C)	35	5.22	6.96	10.86	1.18	1.25	1.14	1554	1680	2618	3.5	6.0
	40	5.14	7.16	10.54	1.20	1.18	1.08	916	1779	2466	4.0	5.6
	45	4.88	6.67	9.65	0.98	1.12	0.98	1469	1543	2067	3.3	4.6

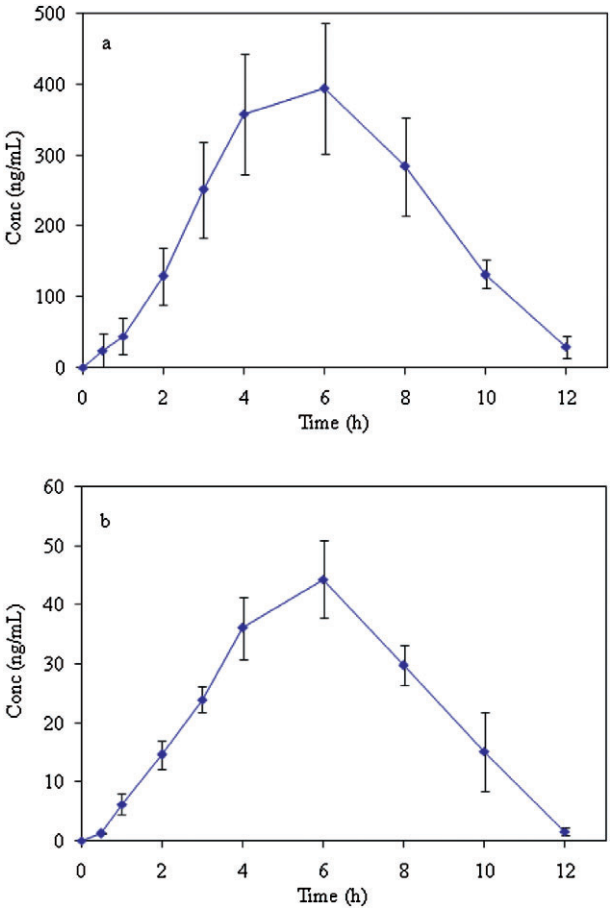

Figure 3. Serum profiles of (a) pioglitazone and (b) felodipine in pigs after administration of buccal tablets, values presented are mean ± SD ($n = 6$).

Table 5. Pharmacokinetic parameters of PIO and FDP after buccal administration to pigs ($n = 6$)

Parameter	Pioglitazone	Felodipine
C_{Max} (ng/mL)	394.6 ± 35.2	44.4 ± 5.2
T_{Max} (h)	5.6 ± 0.8	5.5 ± 0.9
AUC_{0-24} (ng h/mL)	2624.2 ± 314.2	275.8 ± 34.2

1–5000 and 1–4000 ng/mL respectively, in pig serum by HPLC was developed and validated. The method consisted of sample preparation by protein precipitation and extraction into dichloromethane, followed by chromatographic separation and UV detection. No interfering peaks were observed at the elution times of pioglitazone, felodipine and IS. The method was accurate, reproducible, specific and applicable to the evaluation of pharmacokinetic profiles of pioglitazone and felodipine in pigs. Further the method can also be applied for the estimation of pioglitazone and felodipine in human serum after partial validation.

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