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Simultaneous determination of metformin and rosiglitazone in human plasma by liquid chromatography/tandem mass spectrometry with electrospray ionization: Application to a pharmacokinetic study

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Abstract

A selective and sensitive high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (ESI-MS/MS) method for simultaneous determination of metformin and rosiglitazone in human plasma using phenformin as internal standard (IS) has been first developed and validated. Plasma samples were precipitated by acetonitrile and the analytes were separated on a prepacked Phenomenex Luna 5u CN 100A (150 mm × 2.0 mm I.D.) column using a mobile phase comprised of methanol:30 mM ammonium acetate pH 5.0 (80:20, v/v) delivered at 0.2 ml/min. Detection was performed on a Finnigan TSQ triple-quadrupole tandem mass spectrometer in positive ion selected reaction monitoring (SRM) mode using electrospray ionization. The ion transitions monitored were *m/z* 130.27 → 71.11 for metformin, *m/z* 358.14 → 135.07 for rosiglitazone and *m/z* 206.20 → 105.19 for the IS. The standard curves were linear ($r^2 > 0.99$) over the concentration range of 5–3000 ng/ml for metformin and 1.5–500 ng/ml for rosiglitazone with acceptable accuracy and precision, respectively. The within- and between-batch precisions were less than 15% of the relative standard deviation. The limit of detection (LOD) of both metformin and rosiglitazone was 1 ng/ml. The method described is precise and sensitive and has been successfully applied to the study of pharmacokinetics of compound metformin and rosiglitazone capsules in 12 healthy Chinese volunteers.

Keywords: Metformin; Rosiglitazone; Liquid chromatography/tandem mass spectrometry; Pharmacokinetics; Quantitation

1. Introduction

Diabetes is commonly classified into two categories: Type I, insulin-dependent diabetes mellitus. Type II, non-insulin-dependent diabetes mellitus. Type II diabetes is a progressive and complex disease that is difficult to manage effectively in the long-term. For many patients with Type II diabetes, monotheraphy with an oral antidiabetic agent is not sufficient to attain target glycaemic control aims, so combination regimens have become necessary to treat Type II diabetes. Metformin is an oral biguanide antihyperglycemic drug, which increases peripheral insulin sensitivity, inhibits hepatic gluconeogenesis and reduces hepatic glucose production in Type II diabetic patients. Rosiglitazone, a member of the thiazolidinedione class, is a very potent synthetic peroxisome proliferator-activated receptor (PPAR)-γ agonist and effective antidiabetic agent. It exerts its glucose-lowering effects in Type II diabetic patients by increasing insulin sensitivity in target tissues, as well as decreasing hepatic gluconeogenesis. The effect of lowering blood glucose through a combination of metformin and rosiglitazone is significantly better than monotherapy with metformin alone. As an effective treatment for Type II diabetic patients, it is necessary and important to monitor the plasma concentrations of metformin and rosiglitazone and to study their pharmacokinetics in human body for the optimization of dose and dose regimen. Therefore, a sensitive, reliable and rapid method to simultaneously determine metformin and rosiglitazone in human plasma is required.

Several methods have been reported for the determination of metformin and rosiglitazone individually, such as high

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performance liquid chromatography (HPLC) with ultraviolet (UV) detection [1–5] or fluorescence detection [6,7] and capillary electrophoresis (CE) with ultraviolet (UV) detection [8,9]. Most of these methods are tedious and time-consuming involving complex sample preparation, such as equilibrium dialysis [6,7], ultrafiltration [1], solid phase extraction [3] and liquid–liquid extraction [5]. Recently, some new methods for analysis of metformin and rosiglitazone using liquid chromatography/mass spectrometry or tandem mass spectrometry with improved sensitivity and efficiency have been published [10–15]. However, up to now, there is no report on the use of LC/MS/MS method for simultaneous determination of both metformin and rosiglitazone in human plasma. Therefore, this paper describes a simple, selective and highly sensitive method employing one-step protein precipitation for sample preperation and liquid chromatography with electropspray ionisation-tandem mass spectrometry for simultaneous quantitation of both metformin and rosiglitazone in human plasma. This method is validated and has been successfully applied to a pharmacokinetic study of compound metformin and rosiglitazone capsules in healthy Chinese volunteers.

2. Experimental

2.1. Reagents and chemicals

Compound metformin and rosiglitazone capsules (batch no.: 20051214) were obtained from Jiangsu Hengrui Pharmaceutical Corporation (Jiangsu, PR China). Metformin hydrochloride and rosiglitazone reference standards (99.5% and 98.5% purity separately) were supplied by Jiangsu Haosen Pharmaceutical Corporation (Jiangsu, PR China); Phenformin hydrochloride reference standard (internal standard, 99.0% purity) was supplied by Jiangsu Institute for Drug Control (Nanjing, PR China). The chemical structures of metformin hydrochloride, rosiglitazone and phenformin hydrochloride are shown in Fig. 1. HPLC grade methanol and acetonitrile were purchased from VWR (VWR Company, Darmstadt, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

2.2. LC/MS/MS instrument and conditions

The analysis were performed on a Finnigan^{TM} TSQ Quantum Discovery MAX^{TM} LC–MS/MS system equipped with a Finnigan Surveyor LC pump, an auto-sampler and a triple-quadrupole mass spectrometer (Thermo Electron Corporation). The analytical column was a Phenomenex Luna 5u CN 100A (150 mm × 2.0 mm I.D.) and column temperature was set to 40 °C. The mobile phase consisted of methanol:30 mM ammonium acetate pH 5.0 (80:20, v/v) at an isocratic flow rate of 0.2 ml/min. The sample injection volume was 10 μl and run time was 11.0 min. Data acquisition and processing were performed with the Finnigan Xcalibur 2.0 software (Thermo Electron Corporation).

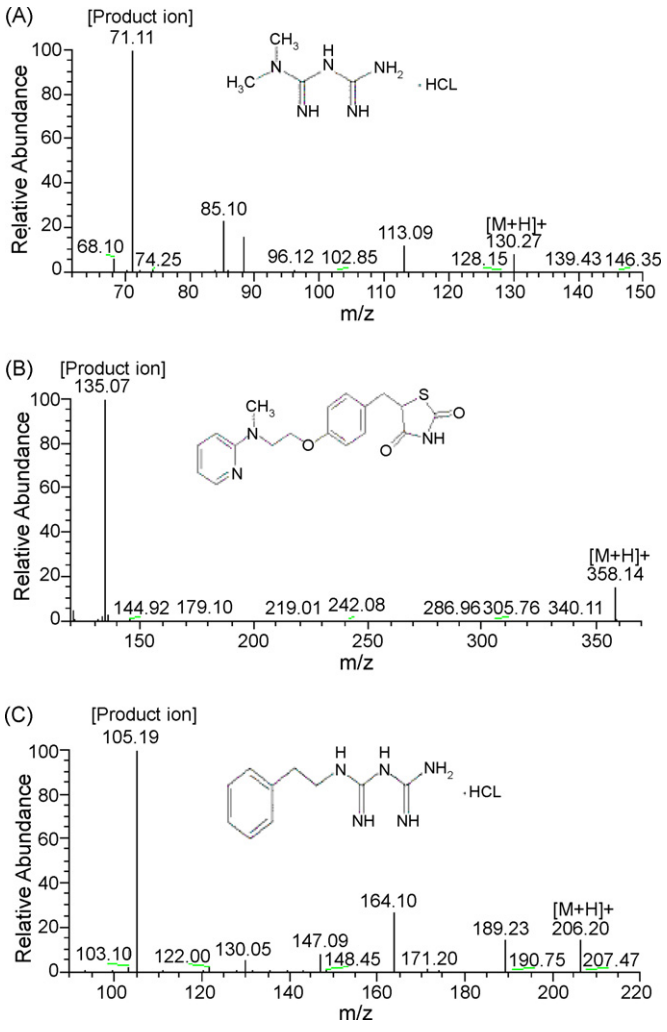

Fig. 1. The structures and product-ion scan spectra of (A) metformin hydrochloride; (B) rosiglitazone; and (C) phenformin hydrochloride.

The mass spectrometer was operated at an electrospray atmospheric pressure ionization source in positive ion mode (ESI$^+$) with selected reaction monitoring (SRM). The sensitivity of SRM was optimized by continuously infusing a solution of 1 μg/ml metformin, rosiglitazone and the IS separately in the mobile phase. The spray voltage was set at 5 kV. The transfer capillary temperature was maintained at 300 °C. The sheath gas and auxiliary gas (nitrogen) pressure were 27 and 5 arb, respectively. The optimized source CID was 10 V. Argon was used as the collision gas with a collision cell gas pressure of 1.5 mtorr (1 torr = 133.3 Pa). The optimized collision energy was 31 eV for metformin, 36 eV for rosiglitazone and 33 eV for the IS. On the basis of the full-scan mass spectra of the analytes, the most abundant ions were selected and the mass spectrometer was set to monitor the transitions of the precursors to the product ions as follows: *m/z* 130.27 → 71.11 for metformin, *m/z* 358.14 → 135.07 for rosiglitazone, and *m/z* 206.20 → 105.19 for the IS. The scan width for SRM was 0.01 *m/z* and scan time was 0.2 s. The peak width settings (FWHM) for both Q1 and Q3 were 0.7 *m/z*.

2.3. Stock solutions

All concentrations of metformin and phenformin refer to the free bases. The stock standard solutions of metformin and rosiglitazone were prepared at 1 mg/ml with redistilled water and methanol, respectively. A stock standard solution of phenformin (IS) at 1 mg/ml was also prepared in redistilled water. All these solutions were found to be stable at 4 °C for 2 months.

Working solutions of metformin were prepared daily in redistilled water by appropriate dilution at 100, 300 ng/ml and 1,2, 4, 10, 20, 40 and 60 μg/ml. The stock solution of rosiglitazone was serially diluted daily with methanol to obtain working solutions at concentrations of 30, 100,200 and 400 ng/ml and 1, 2, 4 and 10 μg/ml. And the stock solution of phenformin was further diluted with redistilled water to prepare the working internal standard solution containing 1 μg/ml of phenformin.

2.4. Sample preparation

To 200 μl plasma in a 1.5-ml polypropylene microcentrifuge tube, 20 μl of the IS working solution (1 μg/ml) and 0.5 ml of acetonitrile were added. The mixture was vortex-mixed thoroughly for 2 min and then centrifuged at 13772 × *g* for 5 min. The supernatant was transferred to another clean tube and centrifuged again at 13772 × *g* for 8 min. Then an aliquot of 10 μl supernatant was directly injected into the HPLC/ESI-MS/MS system.

2.5. Calibration curve and quality control samples

Calibration curves were prepared by spiking blank plasma with 10 μl of one of above mentioned metformin or rosiglitazone working solutions to produce 5, 15, 50, 100, 200, 500, 1000, 2000 and 3000 ng/ml for metformin and 1.5, 5, 10, 20, 50, 100, 200 and 500 ng/ml for rosiglitazone. The following steps were the same as that described in the section of "sample preparation". The blank plasma sample was also analyzed in each run to confirm absence of interferences, but the results for blank samples were not used as part of the calibration curves. Calibration curves were constructed by plotting peak area ratio (y) of metformin or rosiglitazone to the internal standard versus metformin or rosiglitazone concentrations (x) and fitted to the equation $y = bx + a$ by weighted linear regression ($1/\chi^2$ for both metformin and rosiglitazone). Quality control samples, which were analyzed at the same time with test samples, were prepared at concentrations of 15 (low level), 1000 (middle level) and 3000 ng/ml (high level) for metformin and 1.5 (low level), 100 (middle level) and 500 ng/ml (high level) for rosiglitazone in the same manner. The total amount of the quality control samples was about 5% of the test samples in the same batch.

2.6. Method validation

The method was validated for specificity, accuracy, precision, sensitivity, calibration curve and reproducibility according to the FDA guideline [16] for validation of bioanalytical methods.

The specificity of this method was investigated by preparing and analysing six individual human blank plasma samples. Specificity was assessed by comparing the chromatograms obtained from the sample spiked with a concentration of metformin and rosiglitazone at LLOQ with those obtained from blank smaples. Each blank sample was also tested for the visible interference.

When mass spectrometer was used as detector, a matrix effect by ionization competition between the analytes and co-eluents may possibly exist. The potential matrix effect was evaluated by comparing the peak area of the analytes dissolved in the supernatant of the processed blank plasma to that of standard solutions at the same concentration. Three different concentration levels of metformin (15, 1000 and 3000 ng/ml) and rosiglitazone (1.5, 100 and 500 ng/ml) were evaluated by analyzing five samples at each set (every sample was prepared by one source of plasma). The matrix effect of internal standard (100 ng/ml in plasma) was evaluated in the same way.

The within-batch precision and accuracy was determined by repeated analysis of five spiked samples of metformin and rosiglitazone at each QC level in one day ($n = 5$). Between-batch precision and accuracy was determined by repeated analysis in five different days ($n = 5$ series per day, $n = 25$ in total). The concentration of each sample was calculated by standard curve prepared and analyzed in the same day. The precision was defined as the RSD (%) (relative standard deviation) and the accuracy was expressed as a percentage of the measured concentration over the theoretical concentration. The acceptance criteria for within- and between-batch precision were within 15% and accuracy did not exceed 15%.

The limit of detection (LOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively. Each back-calculated concentration standard should meet the following acceptable criteria: no more than 20% deviation at LLOQ and no more than 15% deviation above LLOQ.

The absolute extraction recoveries of metformin and rosiglitazone were determined by comparing the peak area of extracted samples spiked with known amount of the analytes with the peak area of post-extraction blank plasma samples spiked at corresponding concentrations. This procedure was repeated for the three different concentration levels of 15, 1000 and 3000 ng/ml for metformin and 1.5, 100 and 500 ng/ml for rosiglitazone.

Stability experiments were performed to evaluate the stability of metformin and rosiglitazone in stock solution and in plasma samples under different temperature and timing conditions. The freeze and thaw stability was performed by testing three concentration levels of QC plasma samples after three freeze (−20 °C) and thaw (room temperature) cycles. The short-term temperature stability was evaluated by determining QC plasma samples, which were kept at room temperature for a period that exceeded the routine preparation time of samples (around 6 h). The long-term stability was assessed by analyzing QC plasma samples kept at low temperature (−20 °C) for a period of 3 weeks. The post-preparative stability was measured

by reanalyzing extracted QC samples kept under the autosampler conditions (15 °C) for 48 h. The working solution stability of metformin, rosiglitazone and the IS were evaluated at room temperature for 6 h, respectively.

A standard curve in each analytical run was used to calculate the concentration of metformin and rosiglitazone in the unknown samples in the run. It was prepared along with the unknown samples in the same batch and analyzed in the middle of the run. The QC samples in five duplicates at three concentrations (15, 1000 and 3000 ng/ml for metformin and 1.5, 100 and 500 ng/ml for rosiglitazone) were prepared and analyzed with processed test samples at intervals per batch.

2.7. Clinical study method

The method validated above was successfully applied to a pharmacokinetic study of compound metformin and rosiglitazone capsules in healthy Chinese volunteers. The study was approved by Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College. After assessment of medical history, physical examination, electrocardiogram and standard laboratory biochemical examination (blood cell count, biochemical profile and urinalysis), twelve healthy volunteers (6 males and 6 females) were selected to participate in this study. The age of these volunteers ranged from 19 to 30 years (23 ± 3 years), the height ranged from 155 to 178 cm (165 ± 8 cm), and the weight ranged from 48 to 75 kg (59 ± 8 kg). Informed consent was obtained from all the volunteers who had been told the aim and risks of the study. The volunteers were also required to stop taking any medicine at least two weeks before the study.

This study was a single-dose, open-label, randomized, complete three-way crossover study. Every subject was orally administrated the following doses: 250 mg of metformin and 1 mg of rosiglitazone, 500 mg of metformin and 2 mg of rosiglitazone, 1000 mg of metformin and 4 mg of rosiglitazone in the first, second and third period, respectively. Washout period was one week. After fasting 10 h, volunteers were orally administrated an assigned doses with 200 ml of water, and 2 h later, were provided with regular standardized meals. Venous blood samples (3.5 ml) were immediately collected into heparinized tubes before dose and after dose at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, 12, 15 and 24 h. Plasma was separated by centrifugation at $1600 \times g$ for 5 min, and the aliquot of plasma were transferred to another tube and kept frozen at −20 °C until analysis.

The pharmacokinetic parameters were calculated by Program Package (DAS2.0) software. The maximum plasma concentration (C_{max}) and the time point of maximum plasma concentration curve (T_{max}) were directly obtained from the observed concentration versus time profiles. The area under the plasma concentration–time curve from zero hour to the last measurable concentration ($AUC_{0\text{-}\tau}$) and to infinity ($AUC_{0\text{-}\infty}$) was calculated according to the linear trapezoidal rule. The half-time of drug elimination ($t_{1/2}$) was calculated as $0.693/\lambda_Z$, where λ_Z was the elimination rate constant.

3. Results and discussion

3.1. IS selection

Choosing a suitable IS is an important aspect to obtain accuracy when MS/MS is used as detector where matrix effects may lead to inaccuracy of analytical results. Several compounds were investigated to find a suitable IS and finally phenformin hydrochloride was selected as the internal standard in our analysis because of its similar structure, retention action, ionization and extraction with metformin hydrochloride. There was no significant direct ionization suppression between the analytes and the IS throughout the LC/MS/MS study.

3.2. Sample preparation

Metformin had a very high polarity, so it was impossible to extract it from biological fluids using liquid–liquid extraction method. The ion pair solid phase extraction was proved to be feasible for metformin but the procedure was tedious and time-consuming. In the end, a protein precipitation method was selected. To obtain high extraction efficiency, three different protein precipitation agents, acetonitrile, methanol and 20% perchloric acid, were investigated. It was found that methanol caused a marked decrease in mass spectral response to the analytes. Perchloric acid also decreased the mass spectral response and corroded ion source. While acetonitrile did not significantly affect the mass response compared to the other protein precipitation agents. Based on that of Wang et al. [13], acidification precipitation with acetic acid and washing supernatant with dichloromethane were also investigated. But this procedure did not significantly improve extraction efficiency. Finally, a simple single-step acetonitrile protein precipitation was adopted. High extraction efficiency achieved too when this procedure was applied to rosiglitazone. The peak shapes of both metformin and rosiglitazone were better without visible endogenous interference at the retention times.

3.3. Mass spectrometry and chromatography

In this study, ESI was selected as the ionization source. And the mass spectrometer was tuned in both positive and negative ionization modes to check for optimum response of metformin, rosiglitazone and the IS. It was found that the signal intensity of positive ion was higher than negative ion. In the precursor ion full-scan spectra, the most abundant ions were protonated molecules $[M+H]^+$ at *m/z* 130.27 for metformin, *m/z* 358.14 for rosiglitazone and *m/z* 206.20 for the IS. The product-ion scan spectrum showed high abundance fragment ions at *m/z* 71.11 for metformin, *m/z* 135.07 for rosiglitazone and *m/z* 105.19 for the IS, respectively. The ion transitions of *m/z* $130.27 \rightarrow 71.11$ for metformin, *m/z* $358.14 \rightarrow 135.07$ for rosiglitazone and *m/z* $206.20 \rightarrow 105.19$ for the IS were chosen for selected reaction monitoring (SRM). Other main mass spectrometry parameters, such as spray voltage, capillary temperature, sheath gas and auxiliary gas pressure, scour CID, collision gas pressure and collision energy, were optimized by continuous infusion

of a standard solution of metformin (1 μg/ml), rosiglitazone (1 μg/ml) and the IS (1 μg/ml) using a TSQ Quantum electronically controlled integrated syringe and the TSQ Quantum Tune program. The product-ion spectra of these compounds are shown in Fig. 1.

Because of their different polarity, metformin tends to have different retention pattern than rosiglitazone. Using reverse-phase columns, such as C18 column, rosiglitazone had a suitable retention time while metformin had a poor retention property. Thus, a polar column, Phenomenex Luna 5u CN, was adopted. To optimum chromatography conditions, various ammonium acetate buffer concentrations and pH values were investigated. The retention times of metformin and rosiglitazone decreased with the increase in the concentration of ammonium acetate buffer. When decreasing the pH values, the retention time of rosiglitazone increased while metformin decreased. Different ratios of organic phase also affected the retention of metformin and rosiglitazone significantly. Increasing the ratio of organic phase would prolong the retention of metformin and shorten the retention of rosiglitazone. Based on these results, a mobile phase consisting of methonal:30 mmol ammonium acetate pH 5.0 (80:20, v/v) was adopted. The retention times of metformin, rosiglitazone and the IS were 6.3, 3.2 and 9.7 min, respectively, which avoided the interference of ionization between them. The total analysis time was 11 min per sample.

3.4. Assay performance

Selectivity is the ability of the method to distinguish and quantify the analytes in the presence of endogenous interferences. The representative chromatograms of blank plasma, plasma spiked with the analytes and the IS and a volunteer sample collected after oral dosing with test capsules are shown in Fig. 2(A, B and C). No significant interferences from the endogenous plasma components were observed in the retention times of the analytes and the IS.

All the ratios of the peak area of the analytes dissolved in the supernatant of processed blank plasmas compared to that of standard solutions at the same concentration levels were between 85% and 115%, which meant that no significant matrix effect for the analytes and the IS was implied in the method.

Assay linearity was evaluated by calibration curves ranging from 5 to 3000 ng/ml for metformin and 1.5 to 500 ng/ml for rosiglitazone. Using linear regression analysis, an excellent

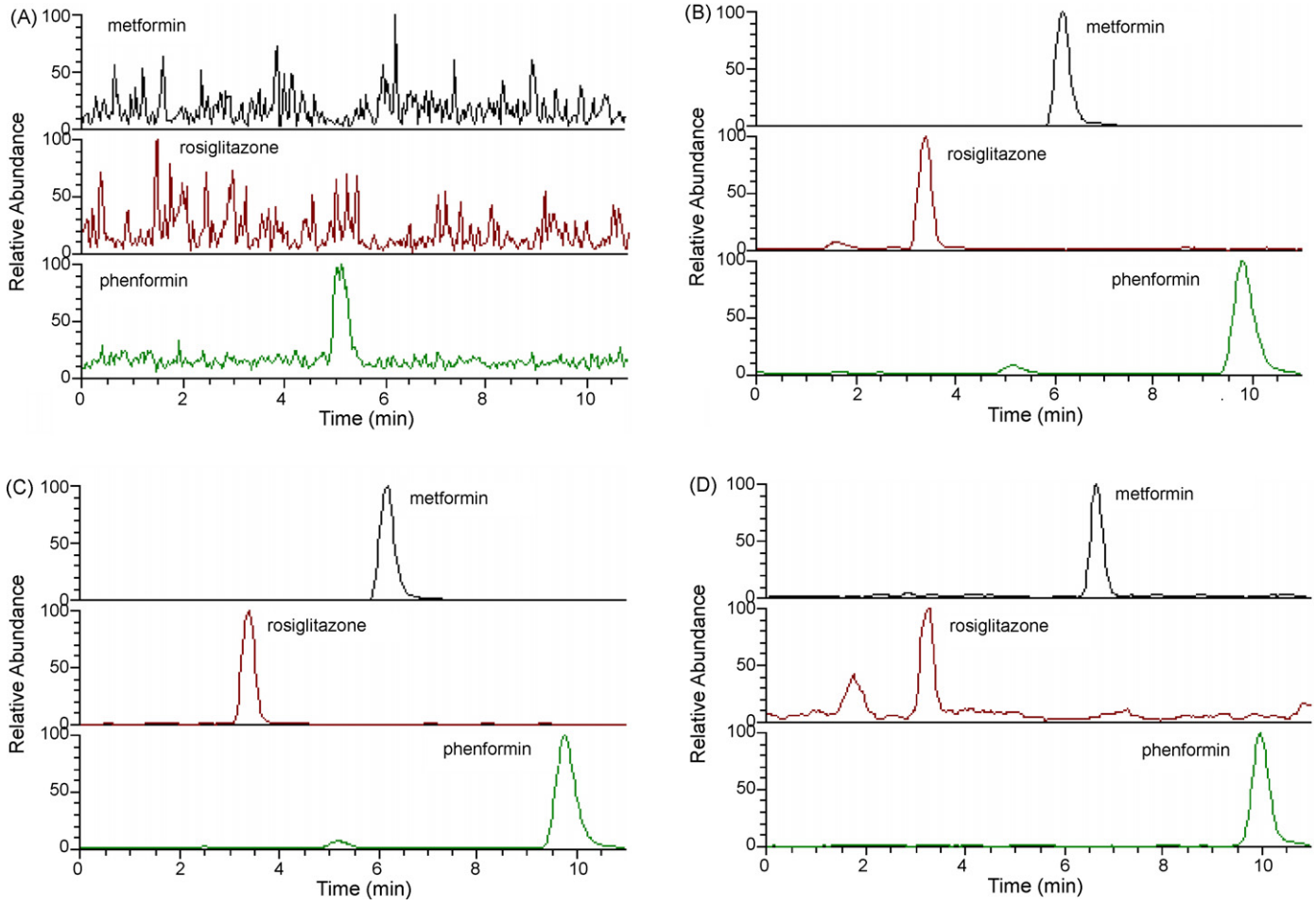

Fig. 2. Representative SRM chromatograms of metformin, rosiglitazone and phenformin (IS) in human plasma. (A) a blank plasma sample; (B) plasma sample spiked with 200 ng/ml metformin, 20 ng/ml rosiglitazone and 100 ng/ml IS; (C) plasma sample from a volunteer (measured metformin and rosiglitazone concentration: 775 and 26 ng/ml); (D) plasma sample at LLOQ (5 ng/ml for metformin and 1.5 ng/ml for rosiglitazone).

Table 1
Precision and accuracy data of back-calculated concentration of calibration samples for metformin and rosiglitazone in human plasma

Metformin				Rosiglitazone			
Concentration added (ng/ml)	Concentration found (mean ± SD, $n=5$) (ng/ml)	Precision (%)	Accuracy (%)	Concentration added (ng/ml)	Concentration found (mean ± SD, $n=5$) (ng/ml)	Precision (%)	Accuracy (%)
5	5.03 ± 0.98	19.6	100.6				
15	14.64 ± 2.38	16.2	97.6	1.5	1.50 ± 0.14	9.2	100.0
50	50.04 ± 6.65	13.3	100.1	5	5.13 ± 0.45	8.8	102.6
100	105.20 ± 13.90	13.2	105.0	10	9.55 ± 1.35	14.1	95.5
200	196.72 ± 27.06	13.8	98.4	20	19.97 ± 1.09	5.5	99.8
500	502.35 ± 70.88	14.1	100.5	50	48.26 ± 6.82	14.1	96.5
1000	1034.83 ± 105.26	10.2	103.5	100	98.01 ± 11.75	12.0	98.0
2000	1910.31 ± 205.92	10.8	95.5	200	198.42 ± 17.69	8.9	99.2
3000	2944.75 ± 139.59	4.7	98.2	500	536.33 ± 56.88	10.6	107.3

linear relationship between peak area ratio and concentrations was exhibited for metformin, $y=0.004509x+0.02118$, $r=0.9995$ and for rosiglitazone, $y=0.01921x+0.0007245$, $r=0.9992$, where y refers to the peak area ratio of metformin or rosiglitazone to the IS and x corresponds to the concentration of metformin or rosiglitazone added into plasma. The weighting factor of both metformin and rosiglitazone was $1/\chi^2$. Mean results of five standard curves for metformin and rosiglitazone are summarized in Table 1.

The LLOQ is defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision. Fig. 2 (D) depicts a representative ion chromatogram for the LLOQ, 5 ng/ml for metformin and 1.5 ng/ml for rosiglitazone. The precision and accuracy at these concentration levels were 19.6% and 100.6% for metformin and 9.2% and 100.0% for rosiglitazone. The LOD of both metformin and rosiglitazone was 1 ng/ml.

The within- and between-batch precision and accuracy data for metformin and rosiglitazone are shown in Table 2. The within-batch precision ranged from 6.7% to 9.5% for metformin and from 5.0% to 6.5% for rosiglitazone, while the between-batch precision ranged from 6.9% to 9.4% for metformin and from 5.7% to 8.2% for rosiglitazone. The within-batch accuracy was between 102.6% and 106.6% for metformin and between 103.8% and 106.9% for rosiglitazone, while the between-batch accuracy was between 94.6% and 106.7% for metformin and between 105.0% and 106.7% for rosiglitazone.

The recoveries of metformin and rosiglitazone were consistent, precise and reproducible. The mean recoveries of the three concentration levels (15, 1000 and 3000 ng/ml for metformin and 1.5, 100 and 500 ng/ml for rosiglitazone) were 77.9%, 80.0% and 87.4% for metformin and 80.2%, 83.6% and 80.3% for rosiglitazone, separately, whereas the RSD were 7.54%, 6.51% and 5.16% for metformin and 6.54%, 6.00% and 2.47% for rosiglitazone, respectively.

Table 3 summarizes the results of the short-term stability, long-term stability, post-preparative stability and freeze and thaw stability of metformin and rosiglitazone. The data showed the reliable stability behavior of metformin and rosiglitazone under the condition tested. Based on the data obtained, the working solutions of metformin, rosiglitazone and the IS were intact within 6 h.

3.5. Applicability in pharmacokinetic study

The method was applied to a pharmacokinetic study of compound metformin and rosiglitazone capsules in 12 healthy Chinese volunteers. The mean plasma concentration log C–t curves of metformin and rosiglitazone are shown in Fig. 3. The pharmacokinetic parameters are presented in Table 4. The results

Table 2
Precision and accuracy of the method for metformin and rosiglitazone in human plasma

Concentration added (ng/ml)	Winthin-batch ($n=5$)			Bentween-batch ($n=5$)		
	Concentration found (mean ± SD) (ng/ml)	Precision (%)	Accuracy (%)	Concentration found (mean ± SD) (ng/ml)	Precision (%)	Accuracy (%)
Metformin						
15	15.99 ± 1.52	9.5	106.6	15.89 ± 1.50	9.4	105.9
1000	1058.25 ± 70.63	6.7	105.8	1066.47 ± 73.23	6.9	106.7
3000	3077.08 ± 217.52	7.1	102.6	2837.92 ± 211.21	7.4	94.6
Rosiglitazone						
1.5	1.60 ± 0.10	6.5	106.9	1.59 ± 0.13	8.2	106.0
100	103.81 ± 6.72	6.5	103.8	105.01 ± 6.02	5.7	105.0
500	533.94 ± 26.84	5.0	106.8	533.59 ± 35.87	6.7	106.7

Table 3
Stability of metformin and rosiglitazone in human plasma

	Metformin				Rosiglitazone			
	Concentration added (ng/ml)	Concentration found (mean ± SD) (ng/ml)	Precision (%)	Accuracy (%)	Concentration added (ng/ml)	Concentration found (mean ± SD) (ng/ml)	Precision (%)	Accuracy (%)
Short-term stability (6 h, room temperature)	15	15.81 ± 1.67	10.5	105.4	1.5	1.55 ± 0.15	9.4	103.7
	1000	1045.32 ± 103.79	9.9	104.5	100	103.13 ± 7.91	7.7	103.1
	3000	2972.90 ± 151.40	5.1	99.1	500	527.23 ± 36.55	6.9	105.5
Freeze and thaw stability (3 cycles, −20 °C–room temperature)	15	16.03 ± 1.62	10.1	106.9	1.5	1.64 ± 0.12	7.1	109.6
	1000	1088.95 ± 54.81	5.0	108.9	100	105.97 ± 6.94	6.6	106.0
	3000	2681.83 ± 73.00	2.7	89.4	500	548.86 ± 33.03	6.0	109.8
Long-term stability (3 weeks, −20 °C)	15	16.58 ± 1.26	7.6	110.5	1.5	1.48 ± 0.12	8.0	98.8
	1000	1036.63 ± 98.76	9.5	103.7	100	107.49 ± 7.08	6.6	107.5
	3000	3164.49 ± 223.18	7.1	105.5	500	532.99 ± 22.16	4.2	106.6
Post-preparative stability (48 h, 15 °C)	15	16.34 ± 1.30	7.9	108.9	1.5	1.52 ± 0.14	9.1	101.5
	1000	1087.84 ± 67.33	6.2	108.8	100	107.11 ± 2.76	2.6	107.1
	3000	2790.94 ± 145.55	5.2	93.0	500	519.27 ± 43.25	8.3	103.9

Table 4
Pharmacokinetic parameters of 12 health volunteers after oral administration of compound metformin and rosiglitazone capsules

Parameters	Metformin			Rosiglitazone		
	250 mg (po)	500 mg (po)	1000 mg (po)	1 mg (po)	2 mg (po)	4 mg (po)
C_{max} (ng/ml)	560.38 ± 146.27	1039.54 ± 255.12	1777.22 ± 593.94	36.16 ± 13.10	67.53 ± 17.50	128.87 ± 24.41
T_{max} (h)	1.38 ± 0.63	1.42 ± 0.76	1.06 ± 0.71	0.99 ± 0.48	0.94 ± 0.58	1.23 ± 0.76
$T_{1/2}$ (h)	3.91 ± 1.92	3.56 ± 0.88	3.80 ± 0.84	4.97 ± 1.61	4.49 ± 0.88	4.25 ± 1.02
MRT (h)	5.04 ± 0.67	5.15 ± 0.57	5.17 ± 0.84	5.57 ± 0.90	5.04 ± 0.81	5.60 ± 0.66
$Auc_{0\rightarrow 24}$(ng h/ml)	3484.89 ± 1336.86	6576.82 ± 1997.74	11441.98 ± 4960.16	184.44 ± 78.33	309.50 ± 76.52	723.66 ± 163.10
$Auc_{0\rightarrow\infty}$(ng h/ml)	3554.17 ± 1392.91	6653.96 ± 2042.69	11633.17 ± 5128.30	192.86 ± 81.48	324.03 ± 79.76	743.03 ± 179.53

of the single-dose study indicated the linear relationship between AUC and dose ($r = 0.9970$ for metformin and $r = 0.9939$ for rosiglitazone). Other pharmacokinetic parameters, such as MRT, $t_{1/2}$, T_{max}, were of no significant difference between the three dosages, indicating pharmacokinetic linearity of three dosages within the dose range studied. Therefore, a preliminary conclusion could be made that the absorption, distribution, metabolism and excretion of metformin and rosiglitazone in human body coincided with the process of first order kinetics.

Kinetic parameters about single-dose study in male and female volunteers are listed in Table 5, respectively. These parameters are of no statistically significant difference between

Table 5
Pharmacokinetic parameters of different genders of 12 health volunteers after oral administration of compound metformin and rosiglitazone capsules

Parameters	Gender	Metformin			Rosiglitazone		
		250 mg (po)	500 mg (po)	1000 mg (po)	1 mg (po)	2 mg (po)	4 mg (po)
C_{max} (ng/ml)	Male	568.77 ± 184.92	1045.34 ± 286.43	1888.52 ± 482.44	31.09 ± 10.02	57.93 ± 15.83	125.03 ± 21.51
	Female	551.99 ± 112.70	1033.74 ± 247.12	1665.92 ± 716.66	41.23 ± 14.68	77.12 ± 14.21	132.71 ± 28.51
T_{max} (h)	Male	1.25 ± 0.67	1.38 ± 1.00	1.29 ± 0.78	0.76 ± 0.43	0.88 ± 0.59	1.29 ± 1.01
	Female	1.50 ± 0.61	1.45 ± 0.51	0.83 ± 0.61	1.21 ± 0.46	1.00 ± 0.61	1.17 ± 0.49
$T_{1/2}$ (h)	Male	4.33 ± 2.66	3.37 ± 0.61	3.98 ± 0.92	4.68 ± 1.19	4.44 ± 1.01	4.40 ± 0.83
	Female	3.49 ± 0.80	3.75 ± 1.11	3.61 ± 0.79	5.26 ± 2.02	4.54 ± 0.83	4.11 ± 1.24
MRT (h)	Male	4.97 ± 0.57	5.04 ± 0.60	5.51 ± 0.99	5.48 ± 1.18	5.04 ± 0.95	5.58 ± 0.31
	Female	5.10 ± 0.81	5.25 ± 0.57	4.83 ± 0.53	5.65 ± 0.62	5.03 ± 0.73	5.61 ± 0.93
$Auc_{0\rightarrow 24}$(ng h/ml)	Male	3304.79 ± 1340.73	6426.80 ± 2202.39	13268.77 ± 5109.67	148.94 ± 27.17	258.44 ± 59.77	688.11 ± 91.57
	Female	3664.99 ± 1434.04	6726.84 ± 1968.66	9615.18 ± 4473.14	219.93 ± 98.68	360.55 ± 55.26	759.22 ± 217.03
$Auc_{0\rightarrow\infty}$(ng h/ml)	Male	3385.16 ± 1381.77	6489.63 ± 2254.16	13568.77 ± 5375.47	156.76 ± 30.60	271.11 ± 56.61	704.02 ± 92.42
	Female	3723.18 ± 1513.46	6818.30 ± 2008.40	9697.58 ± 4468.94	228.95 ± 102.67	376.96 ± 63.79	782.04 ± 242.31

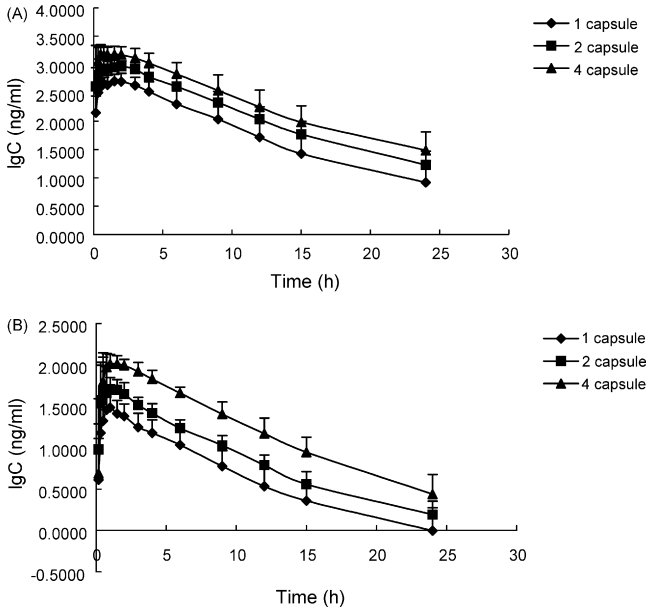

Fig. 3. Mean drug plasma concentration log *C*–*t* curve of metformin (A) and rosiglitazone (B) in 12 volunteers after oral administration of compound metformin and rosiglitazone capsules.

men and women, although some parameters are a little higher or lower in one gender than another. This means that metformin and rosiglitazone are equally absorbed, distributed, metabolized and excreted in both genders. However, further studies may be needed to support this conclusion.

4. Conclusion

The paper first presents a rapid, selective and sensitive LC–MS/MS method using an ESI source in SRM mode for simultaneous determination of metformin and rosiglitazone in human plasma. The basic underlying advantage of this optimized method is that it utilizes only 200 μl plasma sample. And the sample pretreatment is simple single-step acetonitrile protein precipitation without drying or reconstitution. The method was successfully applied to the study of the pharmacokinetics of compound metformin and rosiglitazone capsules in human body. The kinetic parameters obtained from this study can give some sorts of useful information for the development and proper clinical application of the medicine.

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