Simultaneous Determination and Pharmacokinetic Study of Metformin and Rosiglitazone in Human Plasma by HPLC–ESI-MS

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Abstract

A fast, sensitive and specific high-performance liquid chromatography tandem mass spectrometry method was developed for simultaneous determination of metformin and rosiglitazone in human plasma. With phenformin as an internal standard, the analysis was carried out on a C_{18} column (50 mm × 2.1 mm, 3.5 µm) using a mobile phase consisting of acetonitrile–10 mM ammonium acetate (20:80, v/v). The detection was performed by tandem mass spectrometry via electrospray ionization. Linear calibration curves were obtained in the concentration of 1.054–263.5 ng/mL for rosiglitazone and 4.040–5050 ng/mL for metformin. The method was applicable to clinical pharmacokinetic study of metformin and rosiglitazone in healthy volunteers following oral administration.

Introduction

Type 2 diabetes is a long-term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption (1). Treatment of type 2 diabetes (non-insulin dependent) is now possible with orally administered hypoglycemic agents that help to reduce blood sugar levels (2). Currently, oral hypoglycemic drugs prescribed as monotherapy have not provided sufficient hypoglycemic control for type-2 diabetic patients. For this reason, combination therapy is becoming a more prevalent method for achieving satisfactory blood glucose levels (3–7). Metformin hydrochloride is an orally administered biguanide that is widely used in the treatment of type 2 (non-insulin dependent) diabetes mellitus (8,9). It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis commonly found with its analogue, phenformin. Rosiglitazone (I, BRL-49653), an oral antidiabetic agent of the thiazolidinedione class, has received regulatory approval for the treatment of type-2 diabetes as both monotherapy and therapy in combination with other oral antidiabetic agents, due to its advantageous therapeutic profile (10–12). The effect of a combination of metformin and rosiglitazone on lowering blood glucose is significantly better than monotherapy with metformin alone. As an effective treatment for type 2 diabetic patients, it is necessary and important to monitor the plasma concentration of metformin and rosiglitazone and to study their pharmacokinetics in the human body. Therefore, a sensitive, reliable, and rapid method to simultaneously determine metformin and rosiglitazone in human plasma is required.

To date, there has been only one report (13) on the use of liquid chromatography–tandem mass spectrometry (LC–MS–MS) methods for simultaneous determination of metformin and rosiglitazone in human plasma. The lower limits of quantitation (LLOQ) were 5 ng/mL for metformin and 1.5 ng/mL for rosiglitazone, but separation required a long analysis time (11 min). The present paper describes a fast, selective, and highly sensitive approach that enables the determination of metformin at 4.040 ng/mL and rosiglitazone at 1.054 ng/mL with good accuracy and with a total analysis time of 3.5 min. This method has been fully validated and has been applied to a pharmacokinetic study in healthy volunteers after oral administration of metformin and rosiglitazone.

Experimental

Reagents and chemicals

Metformin reference standard (99.2% purity), rosiglitazone (99.3% purity), and phenformin (99% purity) (Figure 1) were

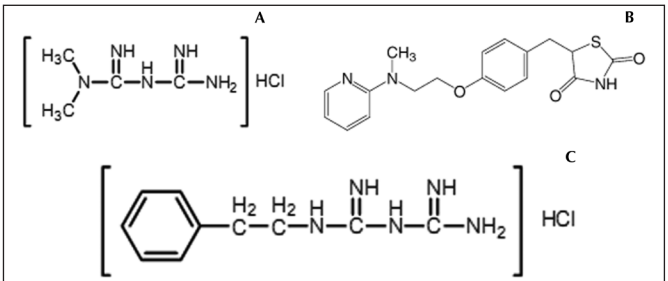

Figure 1. Chemical structure of metformin (A), rosiglitazone (B), and phenformin (IS) (C).

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obtained from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Acetonitrile and ammonium acetate [high-performance [HP] LC grade) were purchased from Dikma (Lake Forest, CA). All other chemicals were analytical grade. Water was purified by redistillation and filtered through 0.22-µm membrane filters before use.

Apparatus and operation conditions

Liquid chromatography

Chromatographic separation was performed on an Agilent 1200 system (Santa Clara, CA) with an autosampler and a column oven that enabled temperature control of the analytical column. A ZorBax Eclipse XDB-C_{18} column (50 mm × 2.1 mm, 3.5 µm) was employed and maitained at room temperature. The mobile phase consisted of acetonitrile–10 mM ammonium acetate (20:80, v/v) at an isocratic flow rate of 0.25 mL/min. The injection volume was 5 µL.

Mass spectrometry

Detection was performed on a Sciex API 4000 Qtrap MS system (Applied Biosystems Sciex, Ontario, Canada) equipped with a Turbo Ionspray interface. Mass spectrometer settings in positive-ion mode (ESI^+) were: voltage at 5000 V, temperature at 400°C, collision gas (N_2) at medium, curtain gas at 20, ion source gases at 40 and 60. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 130.1 → *m/z* 60.1 for metformin, *m/z* 358.2 → *m/z* 134.9 for rosiglitazone and *m/z* 206.3 → *m/z* 59.9 for phenformin. The product ion spectra of metformin, rosiglitazone, and phenformin are shown in Figure 2. Data acquisition and processing were performed with the Analyst software.

Preparation of standards and quality control samples

Standard stock solutions of metformin and phenformin were prepared in water at the concentration of 404.0 µg/mL and 266.0 µg/mL, respectively. A standard stock solution of rosiglitazone was prepared in methanol at the concentration of 421.6 µg/mL. The stock solutions were then serially diluted with water or methanol to provide working standard solutions at the desired concentrations. In addition, the appropriate amount of metformin and rosiglitazone was dissolved in water and methanol to give the final concentrations of 100.5 µg/mL for metformin and 211.2 µg/mL for rosiglitazone for the preparation of quality control (QC) samples. All the solutions were stored at 4°C.

Calibration standards were prepared by spiking 0.2 mL of blank human plasma with working standard solutions of metformin and rosiglitazone. The effective concentrations in standard plasma samples were 4.040, 10.10, 50.50, 101.0, 505.0, 2020, and 5050 ng/mL for metformin and 1.054, 5.270, 10.54, 21.08, 52.07, 105.4, and 263.5 ng/mL for rosiglitazone. One calibration curve was constructed on each analysis day using freshly prepared calibration standards. The quality control samples (QCs) were prepared with blank plasma at LLOQ, low, middle and high concentrations of 4.040, 10.05, 502.5, and 4020 ng/mL for metformin and 1.054, 2.112, 52.80, and 211.2 ng/mL for rosiglitazone. The standards and quality controls were extracted on each analysis day using the same procedures for plasma samples as described later.

Plasma sample preparation

To a 0.2 mL aliquot of plasma sample in 1.5 mL centrifuge tube, 100 µL of internal standard (IS) (1121 ng/mL) and 400 µL acetonitrile were added. The mixture was vortex-mixed thoroughly for 1 min and then centrifuged at 13000 r.p.m. for 10 min. An aliquot of the supernatant was directly injected into the HPLC–MS–MS system.

Method validation

Validation runs were conducted on three consecutive days. Each validation run consisted of a minimum of one set of calibration standards and six replicates of LLOQ and QC plasma samples at three concentrations. The results from LLOQ and QC plasma samples in three runs were used to evaluate the precision and accuracy of the method.

Selectivity

Selectivity was studied by comparing chromatograms of six different batches of blank plasma obtained from six subjects with those of corresponding standard plasma samples spiked with metformin, rosiglitazone and phenformin (2660 ng/mL) and plasma sample obtained after oral doses of metformin and rosiglitazone tablets.

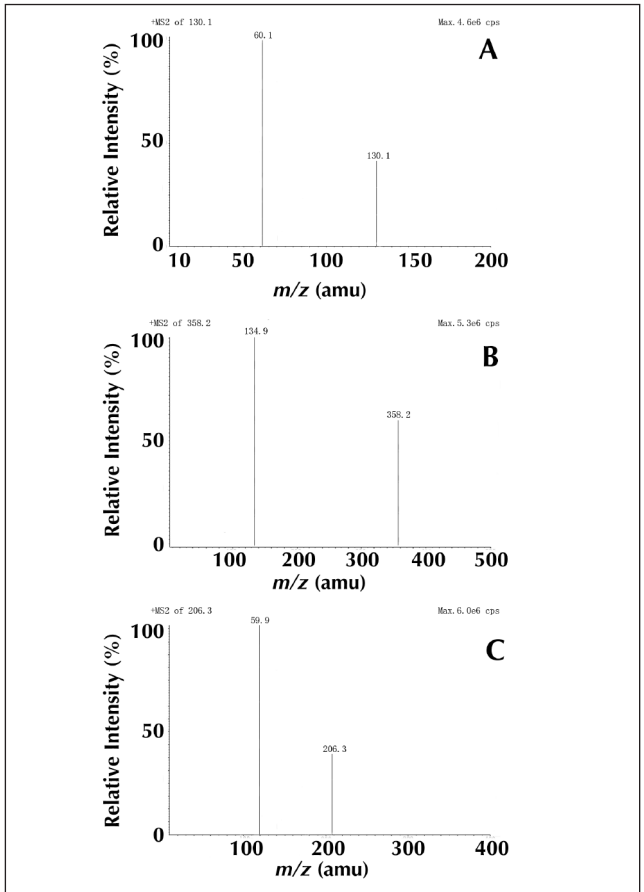

Figure 2. Product ion spectra of metformin (A), rosiglitazone (B), and phenformin (IS) (C).

Linearity and lower limit of quantitation

Calibration curves were prepared by assaying standard plasma samples at seven concentrations of metformin ranging from 4.040–5050 ng/mL and rosiglitazone ranging from 1.054–263.5 ng/mL. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of metformin (or rosiglitazone) to phenformin versus the nominal concentration (x) of metformin (or rosiglitazone). The calibration curves were constructed by weighted ($1/x^2$) least square linear regression.

The LLOQ, defined as the lowest concentration on the calibration curve, was validated using an LLOQ sample for which an acceptable accuracy (RE) within ± 20% and a precision (RSD) below 20% were obtained.

Precision and accuracy

For determination of the intra-day accuracy and precision, a replicate analysis of QC plasma samples of metformin and rosiglitazone was performed on the same day. The run consisted of a calibration curve and six replicates of each LLOQ, low, mid, and high concentration quality control samples. The inter-day accuracy and precision were assessed by analysis of three batches on different days. The precision was expressed as the relative standard deviation (RSD) and the accuracy as the relative error (RE).

Extraction recovery and matrix effect

The recovery was calculated by comparing the peak areas of the metformin and rosiglitazone added into blank plasma and extracted using the protein precipitation procedure with those obtained from the two compounds spiked into post-extraction supernatant at three QC concentration levels. The matrix effect was measured by comparing the peak response of sample spiked post-extraction (A) with that of the standard solution containing equivalent amounts of the two compounds (B). The ratio (A / B × 100) % was used to evaluate the matrix effect. The extraction recovery and matrix effect of the IS were also evaluated using the same method.

Stability

Freeze and thaw stability. The effect of three freeze and thaw cycles on the stability of plasma samples containing metformin and rosiglitazone was determined by subjecting five aliquots of unextracted QC samples at low, mid, and high concentration to three freeze-thaw cycles. After completion of the three cycles, the samples were analyzed and the experimental concentrations were compared with the nominal values.

Long-term stability. Five aliquots of unextracted QC samples at low, mid, and high concentration were stored at –20°C for 20 and 45 days. The samples were then processed and analyzed and the concentrations obtained were compared with the nominal values.

Short-term stability. Five aliquots of unextracted QC samples at low, mid and high concentration were kept at ambient temperature (25°C) for 4 h in order to determine the short-term stability of metformin and rosiglitazone in human plasma. The samples were then processed and analyzed. The concentrations obtained were compared with the nominal values of QC samples.

Post-preparation stability. In order to estimate the stability of metformin and rosiglitazone in the prepared sample, five aliquots of QC samples at low, mid and high concentration were kept in an autosampler maintained at (25°C) for ~ 8 h. The samples were then analyzed, and the concentrations obtained were compared with the nominal values.

Stock solution stability. The stability of stock solution of metformin, rosiglitazone and phenformin were evaluated after 4 h at 25°C and for 30 days at 4°C.

Application to pharmacokinetic study

The method was successfully applied to the pharmacokinetic study of metformin and rosiglitazone in 12 healthy Chinese volunteers (six males and six females). The pharmacokinetic study was a single-dose, open-label, randomized, complete three-way crossover study. Each subject was orally administrated the following doses: 250 mg of metformin and 1 mg of rosiglitazone in the first period, 500 mg of metformin and 2 mg of rosiglitazone in the second period, 1000 mg of metformin and 4 mg of rosiglitazone in the third period. The pharmacokinetic study was approved by the local Ethics Committee and all volunteers gave their signed informed consent to participate in the study according to the principles of the Declaration of Helsinki. Blood samples were collected before and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post-dosing. Samples were centrifuged and plasma was separated and stored at –20°C until analysis.

The maximum plasma concentration (C_{max}) and their time were noted directly. The elimination rate constant (k_e) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693 / k_e$. The area under the plasma concentration-time curve (AUC_{0-t}) to the last measurable plasma concentration (C_t) was calculated by the linear trapezoidal rule. The area under the plasma concentration-time curve to time infinity ($AUC_{0-\infty}$) was calculated as: $AUC_{0-\infty} = AUC_{0-t} + C_t / k_e$.

Results and Discussion

Selection of an IS

The best IS for an LC–MS assay is a deuterated form of the analyte. In our laboratory, no deuterated metformin or rosiglitazone was available; therefore, a compound structurally or chemically similar to the analyte was considered. In LC–MS–MS, the IS should also have similar chromatographic and mass spectrometric behaviors to the analyte, and should mimic the analyte in any sample preparation steps. Phenformin was chosen as the IS for the assay because of its similarity of structure, retention time, and ionization (ESI) to metformin.

Chromatography and mass spectrometry

The separation and ionization of the analytes were affected by the composition of mobile phase. Ammonium acetate was employed to supply ionic strength. With buffers of lower or higher strength than 10 mM, the peak shapes and intensity were unsatisfactory, whereas with 10 mM ammonium acetate there

was an improvement in both peak shape and intensity (Table I). Therefore a mixture of 10 mM ammonium acetate buffer-acetonitrile was finally adopted as the mobile phase.

In order to minimize the run time of the assay, a short C_{18} column was used. The total run time was 3.5 min per sample. The analysis time in the literature (13) was 11 min. The shorter analysis time would better meet the requirements for high sample throughput in bioanalysis.

The LLOQ for metformin was 4.040 ng/mL and 1.054 ng/mL for rosiglitazone. Due to the lower injection volume of 5 µL, the on column sensitivity in our study (the quantity of drug injected on the column per injection) was 20.20 pg for metformin and 5.270 pg for rosiglitazone. Both of these were much lower than the values reported in the literature (13), which were 5 ng/mL for metformin and 1.5 ng/mL for rosiglitazone with the injection volume of 20 µL.

HPLC–MS–MS operation parameters were carefully optimized for determination of metformin and rosiglitazone. The mass spectrometer was tuned in both positive and negative ionization modes with ESI for both metformin and rosiglitazone. Both signal intensity and signal to noise ratio obtained in positive ionization mode were much greater than those in negative ionization mode. In the precursor ion full-scan spectra, the most abundant ions were protonated molecules $(M + H)^+$ *m/z* 130.1, 358.2, and 206.3 for metformin, rosiglitazone and I.S., respectively. The product ion scan spectra showed high abundance fragment ions at *m/z* 60.1, 134.9, and 59.9 for metformin, rosiglitazone and I.S., respectively. MRM using the precursor → product ion transitions of *m/z* 130.1 → *m/z* 60.1, *m/z* 358.2 → *m/z* 134.9 and *m/z* 206.3 → *m/z* 59.9 was employed for quantification of metformin, rosiglitazone, and IS respectively.

Sample preparation

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are techniques often used in the preparation of biological samples as they often improve the sensitivity and robustness of the assay. However, metformin had a very high polarity, so it was impossible to extract it from biological fluids using liquid–liquid extraction method. On the other hand, SPE would not be cost-effective in a high throughput analysis involving many samples. Therefore, in the present experiment, a simple protein precipitation procedure was developed to reduce sample preparation time. No further concentration procedure was needed and the sample preparation procedure was simplified. To test extraction efficiency, three different protein precipitation agents—acetonitrile, methanol, and acetone—were investigated. Acetonitrile had a higher efficiency of precipitation of protein with minimal loss of extracted drug sample. High precipitation efficiency was achieved as well when this procedure was applied to rosiglitazone samples. Ultimately, this simple single-step acetonitrile protein precipitation was adopted.

Table I. Effect of the Concentration of Ammonium Acetate on the Peak Shape and Intensity of Rosiglitazone (52.80 ng/mL)

Conc. of ammonium acetate (mmol/L)	Wh/2 (min)	S/N	Peak intensity (cps)
5	0.25	260	$5.9e^4$
10	0.18	380	$6.6e^4$
20	0.23	300	$6.0e^4$

Method validation

Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. As shown in Figure 3A, no interference from endogenous substance was observed in the retention time of metformin, rosiglitazone or phenformin.

Linearity and LLOQ

The standard calibration curves for metformin and rosiglitazone were linear over the concentration range of 4.040–5050 ng/mL and 1.054–263.5 ng/mL ($r^2 > 0.99$) using weighted least square linear regression analysis with a weigh factor of $1/x^2$. The typical equations for the calibration curves for metformin and rosiglitazone were: $y = 2.760 \times 10^{-4}x + 8.660 \times 10^{-4}$, $r = 0.9999$ and $y = 5.260 \times 10^{-3}x + 7.660 \times 10^{-3}$, $r = 0.9991$.

The lower limit of quantification for metformin and rosiglitazone were 4.040 ng/mL and 1.054 ng/mL with precision and accuracy as presented in Table II with RE within ± 20% and RSD lower than 20%. A typical chromatogram is shown in Figure 3B.

Precision and accuracy

The data of intra-day and inter-day precision and accuracy for the method are listed in Table II. The intra-day and inter-day precision for low, mid, and high QC levels of metformin and rosiglitazone were below 15%, with the accuracy within –7.0% to 3.4%. The precision and accuracy of the present method conforms to the criteria for the analysis of biological samples according to the guidelines of the USFDA, where the precision (RSD) determined at each concentration level is required not to exceed 15% and accuracy (RE) is required to be within ± 15% of the actual value.

Table II. Precision and Accuracy for the Determination of Metformin and Rosiglitazone in Human Plasma

Conc. (ng/mL) metformin		RSD (%)		Relative error (%)
Added	Found	Intra-day	Inter-day	
4.040	3.975 ± 0.1	4.6	12	–1.6
10.05	9.447 ± 0.6	2.3	8.6	–6.0
502.5	517.6 ± 11	1.8	3.4	3.0
4020	4000 ± 96	1.4	4.0	–0.5
Conc. (ng/mL) Rosiglitazone		**RSD (%)**		**Relative error (%)**
Added	**Found**	**Intra-day**	**Inter-day**	
1.054	1.090 ± 0.1	4.0	11	3.4
2.112	1.964 ± 0.1	2.8	1.8	–7.0
52.80	52.17 ± 2.5	1.2	13	–1.2
211.2	206.6 ± 6.3	1.8	6.7	–2.2

*Intra-day: $n = 6$; inter-day: $n = 6$ series per day, 3 days

Extraction recovery and matrix effect

According to the guidelines of USFDA, recovery experiments should be performed at three concentrations (low, medium, and high). This procedure was repeated for five replicates at three concentration levels of QC samples. The extraction recoveries of metformin and rosiglitazone from human plasma at all three QC levels were above 80.0% and the mean extraction recovery of phenformin was 97.9 ± 1.2%.

In terms of matrix effect, all of the ratios previously defined were between 85.0 % and 115.0 %, which means that there was no matrix effect for metformin or rosiglitazone using this method. The recovery and matrix effect data are listed in Table III.

Table III. Results of Recovery and Matrix Effect of Metformin, Rosiglitazone, and Phenformin

Compound	Conc. (ng/mL)	Recovery ± SD (%)	Matrix effect
Metformin	10.05	88.8 ± 1.7	99.5 ± 2.2
	502.5	87.0 ± 0.6	99.5 ± 3.3
	4020	93.6 ± 0.6	95.6 ± 1.3
Rosiglitazone	2.112	84.4 ± 1.2	92.7 ± 7.5
	52.80	80.5 ± 0.4	105.1 ± 2.4
	211.2	92.0 ± 2.6	94.6 ± 1.1
Phenformin (IS)	2260	97.9 ± 1.2	106.3 ± 0.2

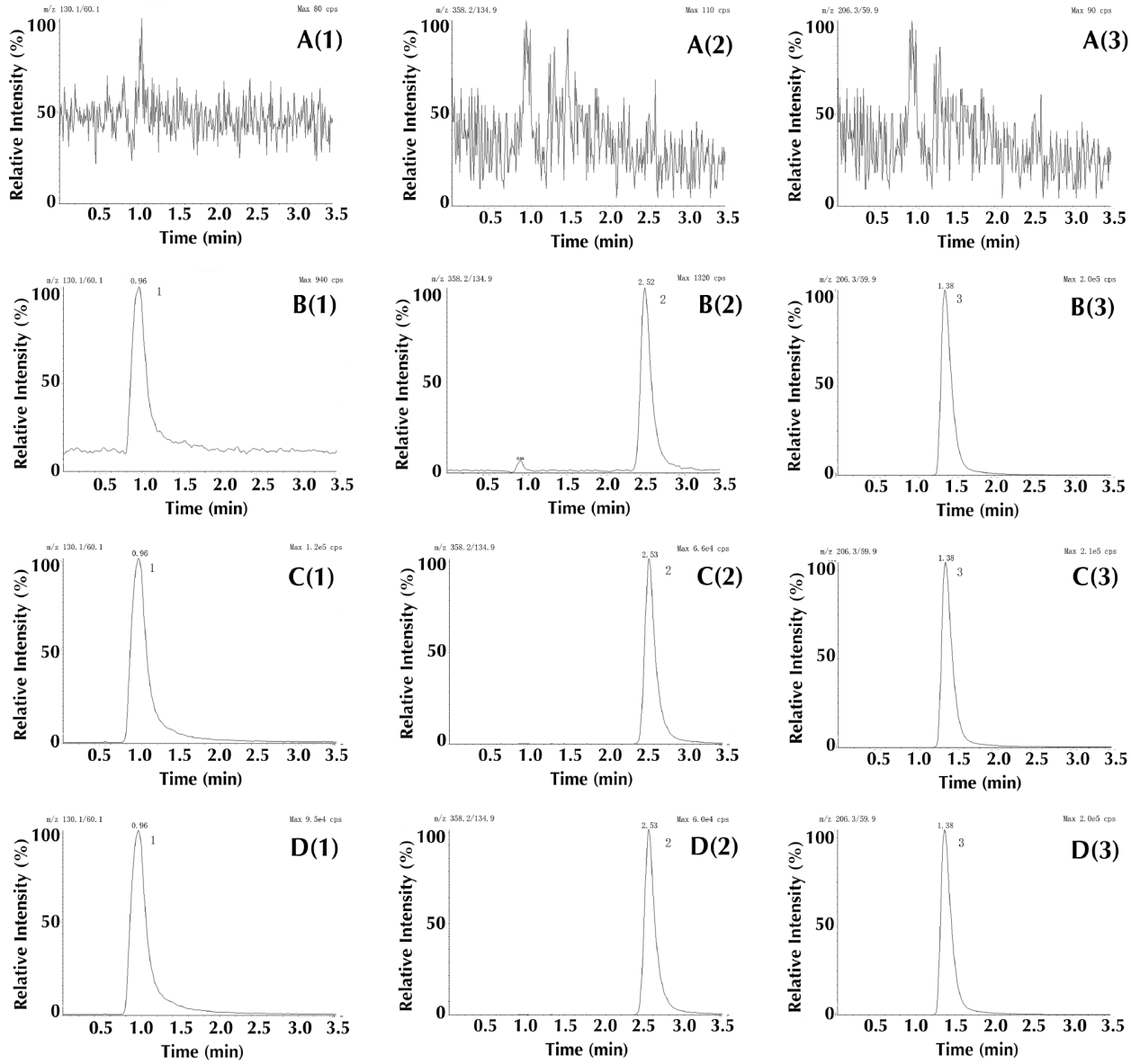

Figure 3. Representative MRM chromatogram of blank plasma samples: metformin (A1), rosiglitazone (A2), and phenformin (A3). Representative MRM chromatogram of a blank plasma sample spiked with: metformin (peak 1) at the LLOQ of 4.040 ng/mL (B1), rodiglitazone (peak 2) at the LLOQ of 1.054 ng/mL (B2), and phenformin (2660 ng/mL, peak 3) (B3). Representative MRM chromatogram of a blank plasma sample spiked with: metformin (peak 1) at 502.5 ng/mL (C1), rosiglitazone (peak 2) at 52.8 ng/mL (C2), and phenformin (peak 3) at 2660ng/mL (C3). Representative MRM chromatogram of a plasma sample from a volunteer 1.0 h after oral administration of metformin and rosiglitazone. The retention time of metformin were 0.96 min (D1). Representative MRM chromatogram of a plasma sample from a volunteer 1.0 h after oral administration of metformin and rosiglitazone. The retention time of rosiglitazone were 2.53 min (D2). Representative MRM chromatogram of a plasma sample from a volunteer 1.0 h after oral administration of metformin and rosiglitazone. The retention time of phenformin were 1.38 min (D3).

Stability

The results from all stability tests presented in Table IV demonstrated a good stability of metformin and rosiglitazone over all steps of the extraction and analytical protocol. The method is therefore proved to be applicable for routine analysis.

Pharmacokinetic application

The present method was successfully applied to a pharmacokinetic study of metformin and rosiglitazone after oral administration in healthy volunteers. Mean plasma concentration-time curves of metformin and rosiglitazone in a single dose study are shown in Figures 4–6. The pharmacokinetic parameters are presented in Table V.

Table IV. Stability of Metformin and Rosiglitazone in Plasma Samples (n = 6)

	Accuracy (mean ± SD) (%)		
Stability metformin	**10.05**	**502.5**	**4020**
Short-term stability	92.5 ± 1.2	103.8 ± 10	101.3 ± 40
Long-term stability	105.7 ± 2.3	112.3 ± 9.6	89.1 ± 62
Freeze-thaw stability	108.7 ± 1.0	105.1 ± 14	104.2 ± 42
Post-preparative stability	107.1 ± 0.5	101.7 ± 8.5	104.0 ± 10
	Accuracy (mean ± SD) (%)		
Stability rosiglitazone	**2.112**	**52.80**	**211.2**
Short-term stability	92.5 ± 0	104.7 ± 0.2	109.5 ± 3.6
Long-term stability	105.4 ± 0.1	99.5 ± 2.7	106.3 ± 1.5
Freeze-thaw stability	106.8 ± 0.2	100.2 ± 3.0	104.1 ± 13
Post-preparative stability	89.7 ± 0	100.9 ± 0.6	104.0 ± 10
Stability of stock solutions	**Accuracy (mean ± SD) (%)**		
	Metformin	**Rosiglitazone**	**Phenformin**
25°C for 4 h	101.2 ± 1.2	98.5 ± 2.0	99.5 ± 3.1
4°C for 30 days	95.8 ± 2.5	105.1 ± 0.5	99.6 ± 0.8

Table V. Pharmacokinetic Parameters of 12 Health Volunteers after Oral Administration of Compound Metformin and Rosiglitazone Tablets

Parameters metformin	**250 mg**	**500 mg**	**1000 mg**
C_{max} (ng/mL)	$925.5 \pm 3.3 \times 10^2$	$1128 \pm 1.6 \times 10^2$	$2473 \pm 1.0 \times 10^3$
T max (h)	1.8 ± 0.6	2.0 ± 1.1	1.9 ± 1.0
$T_{1/2}$ (h)	3.0 ± 0.6	3.5 ± 1.3	3.6 ± 1.0
AUC_{0-t}*	$5522 \pm 1.5 \times 10^3$	$8626 \pm 1.8 \times 10^3$	$1.079 \times 10^4 \pm 2.8 \times 10^3$
$AUC_{0-\infty}$*	$5642 \pm 1.5 \times 10^3$	$8811 \pm 1.8 \times 10^3$	$1.099 \times 10^4 \pm 2.9 \times 10^3$
Rosiglitazone	**1 mg**	**2 mg**	**4 mg**
C_{max} (ng/mL)	167.3 ± 34	208.0 ± 26	355.5 ± 72
T_{max} (h)	1.1 ± 0.3	0.9 ± 0.1	1.6 ± 0.5
$T_{1/2}$ (h)	4.1 ± 0.8	5.0 ± 1.0	5.2 ± 1.8
AUC_{0-t}*	$790.2 \pm 1.2 \times 10^2$	$1412 \pm 4.0 \times 10^2$	$2385 \pm 6.6 \times 10^2$
$AUC_{0-\infty}$*	$806.5 \pm 1.3 \times 10^2$	$1482 \pm 4.7 \times 10^2$	$2542 \pm 8.2 \times 10^2$

* ng.h/mL

Conclusion

A sensitive, selective, and rapid HPLC–MS–MS method for the determination of metformin and rosiglitazone in human plasma is described. Compared with other published methods, the present method offered lower LLOQs of 1.054 ng/mL for rosiglitazone and 4.040 ng/mL for metformin, satisfactory selectivity, and a short run time of 3.5 min. The method has been successfully applied to a pharmacokinetic study of metformin and rosiglitazone administered in tablet form to healthy volunteers.

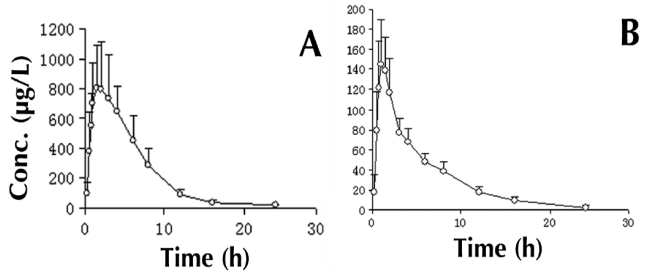

Figure 4. Mean plasma concentration–time curve of metformin in 12 volunteers after a single oral dose of 250 mg (A), and mean plasma concentration–time curve of rosiglitazone in 12 volunteers after a single oral dose of 1 mg (B).

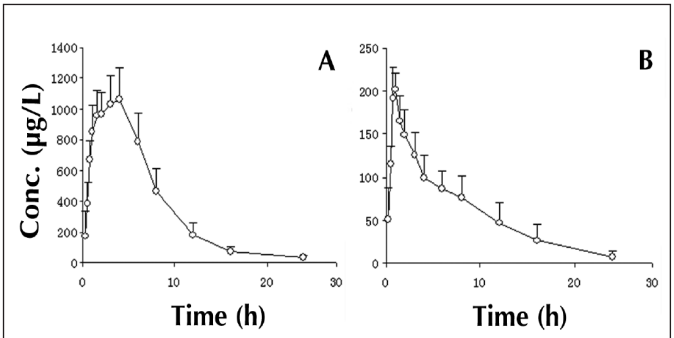

Figure 5. Mean plasma concentration–time curve of metformin in 12 volunteers after a single oral dose of 500 mg (A), and mean plasma concentration–time curve of rosiglitazone in 12 volunteers after a single oral dose of 2 mg (B).

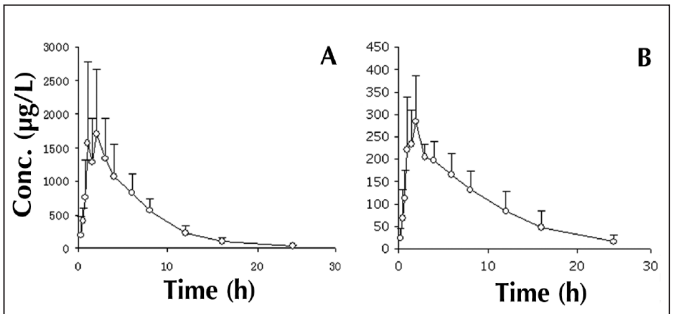

Figure 6. Mean plasma concentration–time curve of metformin in 12 volunteers after a single oral dose of 1000 mg (A), and mean plasma concentration–time curve of rosiglitazone in 12 volunteers after a single oral dose of 4 mg (B).

Acknowledgment

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