Talanta 104 (2013) 109–115

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of glibenclamide and puerarin in rat plasma by UPLC–MS/MS: Application to their pharmacokinetic interaction study

Ning Li, Ying Deng, Dan Wang, Ying Qiao, Famei Li *

Department of Analytical Chemistry, School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 11 July 2012
Received in revised form
12 November 2012
Accepted 17 November 2012
Available online 23 November 2012

Keywords:
Glibenclamide
Puerarin
UPLC–MS/MS
Pharmacokinetics
Rat plasma

ABSTRACT

In the treatment of diabetes mellitus, glibenclamide and puerarin may be co-administered unwittingly or wittingly. An ultra performance liquid chromatography–tandem mass spectrometry method was developed to determine the concentrations of glibenclamide and puerarin in rat plasma for the study of pharmacokinetic interaction between them. Analytes were extracted using liquid–liquid extraction. The separation was achieved on a Waters BEH C_{18} column using 5 mmol/L ammonium acetate solution (containing 0.1% formic acid) and methanol as mobile phase with a linear gradient program. Electrospray ionization source was applied and operated in the multiple reaction monitoring positive mode. The proposed method was proved simple, specific and reliable. Glibenclamide, *Pueraria lobata* extract and glibenclamide in combination with *P. lobata* extract were orally administered to rats, respectively. Pharmacokinetic parameters were estimated by Microsoft Excel software and analyzed by SPSS 12.0 software. Compared with glibenclamide group, pharmacokinetic parameters of glibenclamide in the co-administration group such as area under the curve and mean residence time were increased while clearance was decreased. Pharmacokinetic parameters of puerarin in the co-administration group such as peak concentration and area under the curve were enlarged while clearance and apparent volume of distribution were reduced compared with *P. lobata* extract group. These changes could enhance drug efficacy, but could also make drug accumulation to increase adverse effects, so it was suggested that the dosage should be adjusted or the drug concentration in plasma should be monitored if glibenclamide and puerarin were co-administered.

1. Introduction

Chinese medicines include raw medicinal materials, Chinese proprietary medicines and some dietary supplements composed of raw medicinal materials. They are becoming more and more popular throughout the world due to their natural origin and harmlessness. Despite their mechanisms of action being generally unknown, evidence of efficacy being lack and toxicological data being inadequate, they are still taking an active part in people's health care, prevention and therapy of disease [1,2]. Correspondingly, these Chinese medicines are often co-administered with synthetic drugs in clinical practice unwittingly or wittingly, which may greatly raise the potential of drug–herb interaction. The interaction between Chinese medicines and drugs is a significant safety concern because the pharmacokinetics of drug and/or active constituent of Chinese medicines may be altered by co-administration, severe and perhaps even life-threatening adverse reactions may occur [3–5].

Pueraria lobata (the root of *P. Lobata* (willd.) Ohwi) is widely used in clinical Chinese medicines and also as a food in Asian countries. Puerarin is an active and main constituent of *P. lobata* and it can improve insulin resistance and restore the pathological abnormalities induced by high glucose levels [6,7]. It has also been reported that puerarin or *P. lobata* extract exhibit activity in the treatment of diabetes mellitus [8,9]. Many of Chinese medicines used for diabetes on market contain *P. lobata*. The toxicological information about puerarin is not sufficient, but life-threatening interaction between *P. lobata* extract and methotrexate in rats has been found [10,11]. In addition, both verapamil and erythromycin lactobionate could inhibit the elimination of puerarin in rats [12,13].

Glibenclamide (glyburide) is a second generation oral sulfonylurea anti-diabetic agent widely used for the treatment of type II diabetes mellitus and gestational diabetes mellitus [14,15]. It is rapidly and completely absorbed from the gastrointestinal tract and can play a potent and long-acting anti-diabetic effect [16]. Glibenclamide acts mainly by stimulating endogenous insulin release from beta cells of pancreas. Meanwhile, it has the highest hypoglycemic risk in the orally available insulinotropic drugs and should be taken carefully [17]. Moreover, some drugs or components such as clarithromycin, atorvastatin and 18α-glycyrrhizin

* Corresponding author. Tel./fax: +86 24 2398 6289.
E-mail address: lifamei@syphu.edu.cn (F. Li).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.11.037

were reported to pharmacokinetically interact with glibenclamide and increase the risk by elevated glibenclamide concentration when being co-administered [18–20].

In the treatment of diabetes mellitus, glibenclamide and puerarin may be co-administered clinically. Furthermore, some manufacturers add synthetic anti-diabetic drugs illegally in the herbal products for the sake of excess profits and it was found that glibenclamide was the most common adulterant in our previous study [21]. Therefore, they may be co-administered by patients unwittingly. However, there have been no animal or clinical experiments on the pharmacokinetic interaction between glibenclamide and puerarin. It is therefore necessary to perform a study to compare the pharmacokinetics of glibenclamide and puerarin in rats when being administered orally alone or being co-administered. Although some methods have been developed for measurement of glibenclamide or puerarin in plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS) [22–25], there has been no method reported for simultaneously determining the glibenclamide and puerarin so far.

In this paper, an ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed to determine the concentrations of glibenclamide and puerarin in rat plasma and pharmacokinetic interaction between them was studied to provide some suggestion for clinical practice.

2. Materials and methods

2.1. Chemicals

Reference standards of glibenclamide, puerarin and glipizide (internal standard, IS) were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Pueraria lobata extract (containing 65% puerarin) was supplied by Ankang Hemaidisen Herbal Pharmaceutical Co. Ltd (Ankang, Shanxi, China). Methanol of HPLC grade was purchased from Tedia (Fairfield, OH, USA). Ammonium acetate and formic acid (FA) of HPLC grade were obtained from Dikma (Richmond Hill, NY, USA). Ethyl acetate of analytical grade was from Ruijinte Chemical Co. Ltd (Tianjin, China). Water was purified by redistillation and filtered through 0.22 μm membrane filter before use.

2.2. Animals and administration

Male Wistar rats weighing 260 ± 20 g, SPF grade, were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Throughout the study, the rats were kept in an air-conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of 65 ± 10%, and had access to the standard laboratory food and water. Animal experiments were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Commission of Science Technology of the People's Republic of China.

The rats were fasted overnight but with free access to water before administration. Glibenclamide and *P. lobata* extract were suspended in 0.5% CMC–Na solution, respectively or together. All the rats were randomly divided into three groups, each containing 6 animals. Group A was orally administered with glibenclamide alone at 10 mg/kg, group B was orally administered with *P. lobata* extract alone at 615 mg/kg (equal to 400 mg/kg puerarin), and group C was treated with a mixture of glibenclamide and *P. lobata* extract at the same dosages as above.

2.3. Blood collection and sample preparation

Blood samples (0.3 mL) were collected from the suborbital venous plexus before intragastric gavage and after that at 0.17, 0.33, 0.50, 0.67, 1.0, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12, 24 and 36 h, transferred to heparinized tubes, and centrifuged at 13,000 rpm for 10 min to obtain plasma. Plasma samples were stored at −20 °C until analysis. For sample preparation, an aliquot of 20 μL IS solution was firstly pipetted into glass tubes and then evaporated to dryness under a gentle stream of air at 40 °C. The residue was vortex-mixed vigorously with 100 μL of plasma samples and 50 μL phosphoric acid (0.25 mol/L) for 30 s. Afterward, ethyl acetate (3 mL) was added to each tube and vortex mixed for approximately 3 min in order to facilitate liquid–liquid extraction (LLE). This was followed by centrifugation for 10 min at 3500 rpm to separate the layers. The organic layer was transferred to a clean test tube and dried under a flow of air at 40 °C. The residue was reconstituted with 100 μL of methanol–water (30:70, *v/v*) and an aliquot of 10 μL was subjected to LC–MS/MS analysis.

2.4. UPLC–MS/MS method

The chromatography was performed on an Acquity Ultra Performance LC™ system (Waters Corp., Milford, MA, USA) with cooling autosampler. The separation was achieved on an Acquity UPLC BEH C_{18} column (50 mm × 2.1 mm) with 1.7 μm particle size (Waters Corp.). Gradient elution was applied using methanol (A) and 5 mmol/L ammonium acetate solution containing 0.1% FA (B) as the mobile phase and programmed as below: the gradient started with 30% eluent A, increased linearly up to 70% in 2 min and the composition was held further for 2.5 min before returned to 30% of eluent A immediately, followed by a re-equilibration for 1 min. The flow rate was set at 0.20 mL/min and 10 μL of sample solution was injected with partial loop mode. The column temperature was maintained at 40 °C and the autosampler temperature was kept at 4 °C.

For the MS/MS detection, electrospray ionization (ESI) source was operated in positive ion mode and the optimal ionization source parameters were: capillary voltage 4.0 kV, extractor voltage 2 V, RF lens voltage 0.6 V, source temperature 110 °C and desolvation temperature 500 °C. Nitrogen was used as the desolvation and cone gas with a flow rate of 600 and 50 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 3×10^{-3} mbar. The cone voltage was 25 V for puerarin, 20 V for glipizide and 20 V for glibenclamide. The optimized collision energy for puerarin was 25 eV, glipizide 13 eV and glibenclamide 13 eV. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 417 → 297 for puerarin, *m/z* 446 → 321 for glipizide and *m/z* 494 → 369 for glibenclamide, with scan time of 0.10 s per transition.

2.5. Preparation of calibration standards and quality control samples

A mixed stock solution containing 186 μg/mL of puerarin and 42.8 μg/mL of glibenclamide was prepared in methanol. A series of working standard solutions were prepared by successive dilution of the mixed stock solution with methanol. A 55.5 ng/mL IS working solution was similarly prepared by diluting a stock standard solution of glipizide with methanol. Calibration standards were prepared daily by evaporating 20 μL of working solutions and 20 μL of IS solution to dryness and then fully mixed with 100 μL of blank plasma. The effective concentrations in standard plasma samples were 18.6, 37.2, 74.4, 186, 372, 744, 1.86×10^3, and 3.72×10^3 ng/mL for puerarin and 4.28, 8.56,

17.12, 42.8, 85.6, 171.2, 428, and 856 ng/mL for glibenclamide. Quality control (QC) samples were prepared at 46.5, 297.6, 2976 ng/mL for puerarin and 10.7, 68.48, 684.8 ng/mL for glibenclamide.

2.6. Method validation

Samples of rat blank plasma, blank plasma spiked with the analytes and IS and plasma after gavage were used to evaluate the specificity. Calibration curves were generated by using the peak area ratios of the analytes to IS *versus* concentrations and runned on three separate days. Extraction recovery was determined at three QC levels by comparing the peak areas of analytes between plasma samples spiked with analytes before and after extraction. Matrix effect was evaluated at three QC levels by comparing the peak areas of analytes obtained from plasma samples spiked with analytes after extraction to those of the pure standard solutions at the same concentrations. Extraction recovery and matrix effect of IS were also measured at one concentration level using the same method.

To evaluate the intra- and inter-day precision and accuracy, six replicates of QC samples at three concentrations were analyzed on three consecutive days using calibration curves prepared on the same day. The precision was expressed as relative standard deviation (R.S.D.) and accuracy as relative error (R.E.). The lower limit of quantification (LLOQ) is defined as the lowest concentration of standard that can be measured with an acceptable accuracy and precision ($\leq$20% for both parameters). The limit of detection (LOD) is defined as the lowest amount of analyte that could be detected and determined as the concentration with a signal to noise ratio of 3. Stability of the analytes in rat plasma was assessed by analyzing five replicates of QC samples at three concentrations stored for 4 h at room temperature, three cycles of freezing at -20 °C and thawing and stored for 20 days at -20 °C, respectively. The extracted QC samples kept in the autosampler at 4 °C for 12 h were analyzed to evaluate post-preparation stability.

2.7. Determination of real samples and data analysis

The established UPLC–MS/MS method was used to determine the concentrations of glibenclamide and puerarin in rat plasma after oral administration. The mean plasma concentration–time curves were plotted. All the data was processed by non-compartmental analysis using Microsoft Excel software. The peak plasma concentration (C_{max}) and the time to peak concentration (T_{max}) were obtained from visual observation. The other pharmacokinetic parameters of elimination rate constant (k_e), half life ($t_{1/2}$), area under the curve (AUC), mean residence time (MRT), clearance (Cl/F) and volume of distribution (V_d/F) were calculated based on moment methods. The pharmacokinetic parameters were expressed as the mean $\pm$ S.D. (standard deviation). Statistical comparisons were carried out using the Statistical Package for Social Science software (SPSS), version 12.0 (International Business Machines Corporation—IBM, Armonk, US), differences were considered to be significant when $P<0.05$.

3. Results and discussion

3.1. Analysis method development

Analysis method was set up by optimizing UPLC and MS/MS conditions to obtain the best possible sensitivity. The analytes were analyzed firstly using MS by syringe infusion of individual standard solution and they were all more efficiently ionized in ESI positive mode than in negative mode. ESI positive mode was therefore employed. Parameters such as ESI source temperature, capillary and cone voltage, flow rate of desolvation gas and cone gas were optimized to obtain highest intensity of protonated molecules of analytes. The collision energy of collision-induced decomposition (CID) was optimized for maximum response of the fragmentation of analytes. MRM was used to monitor precursor ion and product ion, which could reduce interference and enhance selectivity. Full-scan product ion spectra of $[M+H]^+$ of puerarin, glipizide and glibenclamide and their fragmentation pathways are shown in Fig. 1.

Puerarin is an isoflavone C-glycoside and glibenclamide is a sulfonylurea compound. A gradient elution was used to shorten analysis time because of variance in polarity between puerarin and glibenclamide. The effect of composition of mobile phase on the analyte response was investigated. It was observed that the presence of methanol in the mobile phase generated higher response compared to acetonitrile. Therefore methanol was used as the organic phase. The response of glibenclamide increased when FA was added into the water and the response of puerarin increased when ammonium acetate was used. Finally, a simple programmed solvent gradient using methanol and 5 mmol/L ammonium acetate solution (containing 0.1% FA) provided the best mobile phase in terms of peak shape, rapidity and sensitivity. Glipizide had a suitable retention time in the chromatographic separation and was used as the IS.

In the pretreatment of plasma sample, protein precipitation (PPT) and LLE are often used. PPT was tested initially with methanol or acetonitrile, but the interference from plasma sample matrix could not be eliminated. Thus LLE of analytes and IS from plasma samples was explored. Ethyl acetate was used as extraction solvent which could extract glibenclamide and puerarin simultaneously. Phosphoric acid (0.25 mol/L), hydrochloric acid (0.10 mol/L) and FA (5%) were added separately into the plasma before liquid–liquid extraction to improve extraction efficiency. The best results were obtained when phosphoric acid was used. The LLE method was simple, rapid and could produce purified samples.

3.2. Analysis method validation

Representative MRM chromatograms of puerarin, glipizide and glibenclamide in rat plasma samples are shown in Fig. 2. The retention time for them was 1.22, 2.93 and 3.67 min, respectively. No interference from endogenous substances was observed at the retention time of analytes and IS. Calibration curves showed good linearity over the range of 18.6–3.72×10^3 ng/mL for puerarin and 4.28–856 ng/mL for glibenclamide with the correlation coefficient (r^2) over 0.99. The mean extraction recoveries at three QC levels were 74.3 $\pm$ 6.6%, 73.4 $\pm$ 5.2% and 71.4 $\pm$ 4.1% for puerarin and 87.4 $\pm$ 3.7%, 89.2 $\pm$ 2.3% and 79.0 $\pm$ 1.7% for glibenclamide. The mean recovery of IS was 98.5 $\pm$ 2.4%. The matrix effects on the analytes and IS were between 85% and 115% indicating that the co-extracted matrix produced no significant effect on the signal intensities.

The data for intra- and inter-day precision and accuracy for puerarin and glibenclamide are summarized in Table 1. The intra- and inter-day precision of puerarin and glibenclamide was less than 13%, and the accuracy was between -8.2% and 8.2%. The LLOQs of puerarin and glibenclamide were 18.6 ng/mL and 4.28 ng/mL, respectively. The LODs were 3.72 ng/mL and 0.856 ng/mL, respectively. All the results of stability tests are summarized in Table 2, which demonstrates the good stability of puerarin and glibenclamide stored at room temperature for 4 h and at -20 °C for 20 days in plasma, during three freeze and thaw cycles, and at 4 °C for 12 h after sample preparation. Therefore, the method has been proved to be applicable for routine analysis.

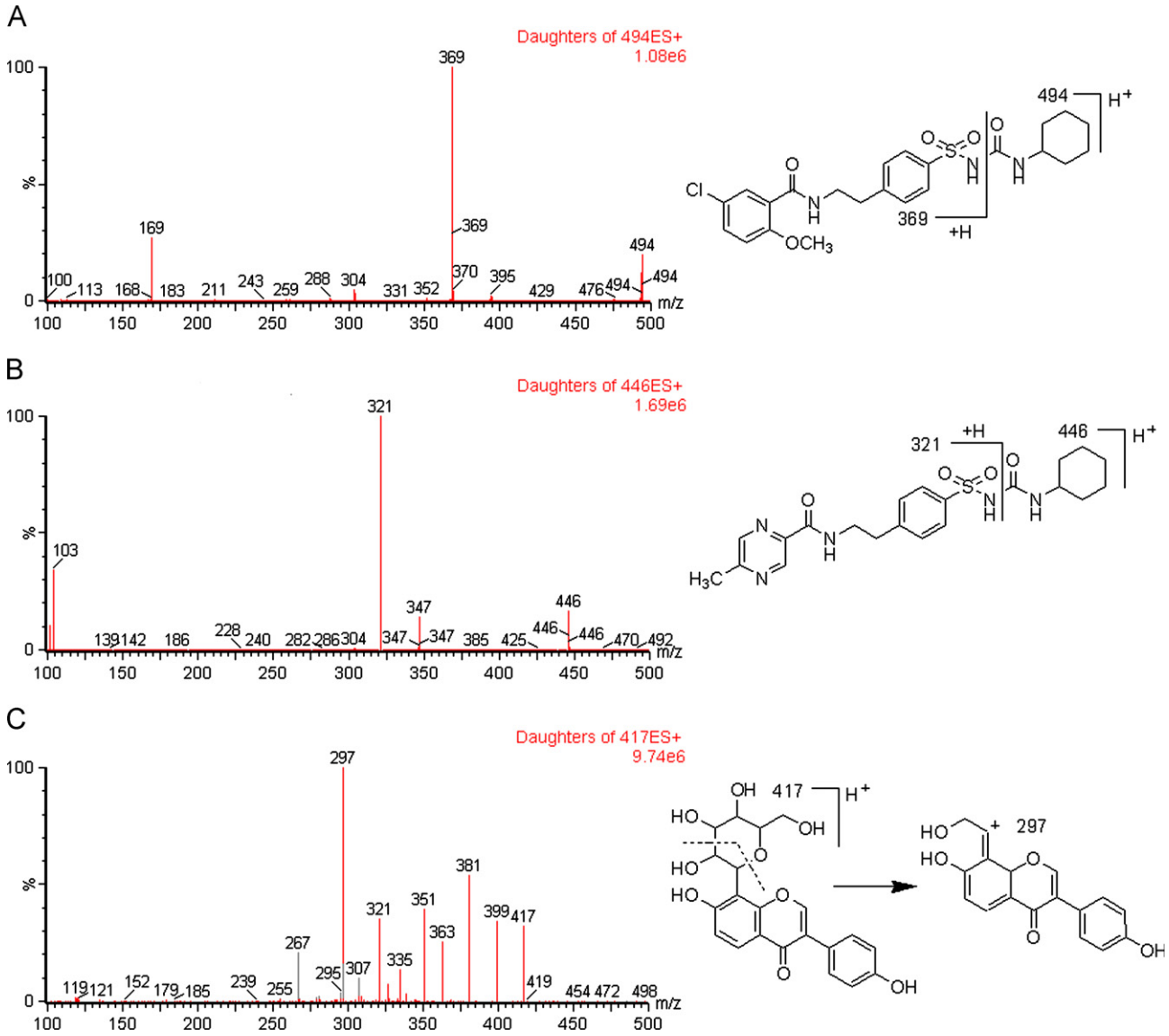

Fig. 1. Full-scan product ion spectra of $[M+H]^+$ of glibenclamide (A), glipizide (B) and puerarin (C) and their fragmentation pathways.

3.3. Pharmacokinetic study

The mean plasma concentration–time curves for glibenclamide and puerarin are presented in Fig. 3. Fig. 3(A) shows the curves for glibenclamide after oral administration of glibenclamide alone (10 mg/kg) and co-administration with *P. lobata* extract (615 mg/kg, equal to 400 mg/kg puerarin). It can be observed that first an increase of the concentration of glibenclamide is then followed by a slow decline (it can still be detected until 36 h after administration). Fig. 3(B) shows the curves for puerarin after oral administration of *P. lobata* extract alone (615 mg/kg, equal to 400 mg/kg puerarin) and co-administration with glibenclamide (10 mg/kg). It can be seen that puerarin is absorbed rapidly and can be detected at 10 min after administration. Table 3 summarizes the pharmacokinetic parameters of glibenclamide found in group A and group C's rats, the pharmacokinetic parameters of puerarin found in group B and group C's rats are listed in Table 4. After co-administration of glibenclamide and *P. lobata* extract, AUC_{0-t}, $AUC_{0-\infty}$, MRT_{0-t} and $MRT_{0-\infty}$ of glibenclamide were significantly increased by 47.9%, 56.0%, 21.8% and 30.4%, respectively, while Cl/F was significantly decreased by 33.3%. On the other hand, C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of puerarin were markedly increased by 59.1%, 52.8% and 51.1%, while Cl/F and V_d/F were markedly decreased by 36.8% and 45.3%, respectively. These results showed that the pharmacokinetics of glibenclamide and puerarin changed when being co-administered.

As with drug–drug interactions, drug–herb interactions may be mediated by physicochemical properties, pharmacokinetic properties and pharmacodynamic properties. Among them, pharmacokinetic properties are the main cause. Pharmacokinetic interaction between Chinese medicine and drug may occur in the phase of absorption, distribution, metabolism or excretion. For most orally-administered drugs, liver is the primary site of metabolism and cytochrome P450s (CYP450) are very important drug-metabolizing enzymes. Previous studies have showed that herbal extract or formula may affect CYP450 and then change the process of drug metabolism [26,27]. Besides, P-glycoprotein (P-gp), a well-know drug transporter, plays an important role in the absorption, distribution, or excretion of drugs, and it can also be induced or inhibited resulting in the interactions based on transport [28,29].

From our results, increase in AUC, MRT and decrease in Cl/F of glibenclamide were observed in presence of *P. lobata* extract. These facts clearly indicated that *P. lobata* extract slowed down

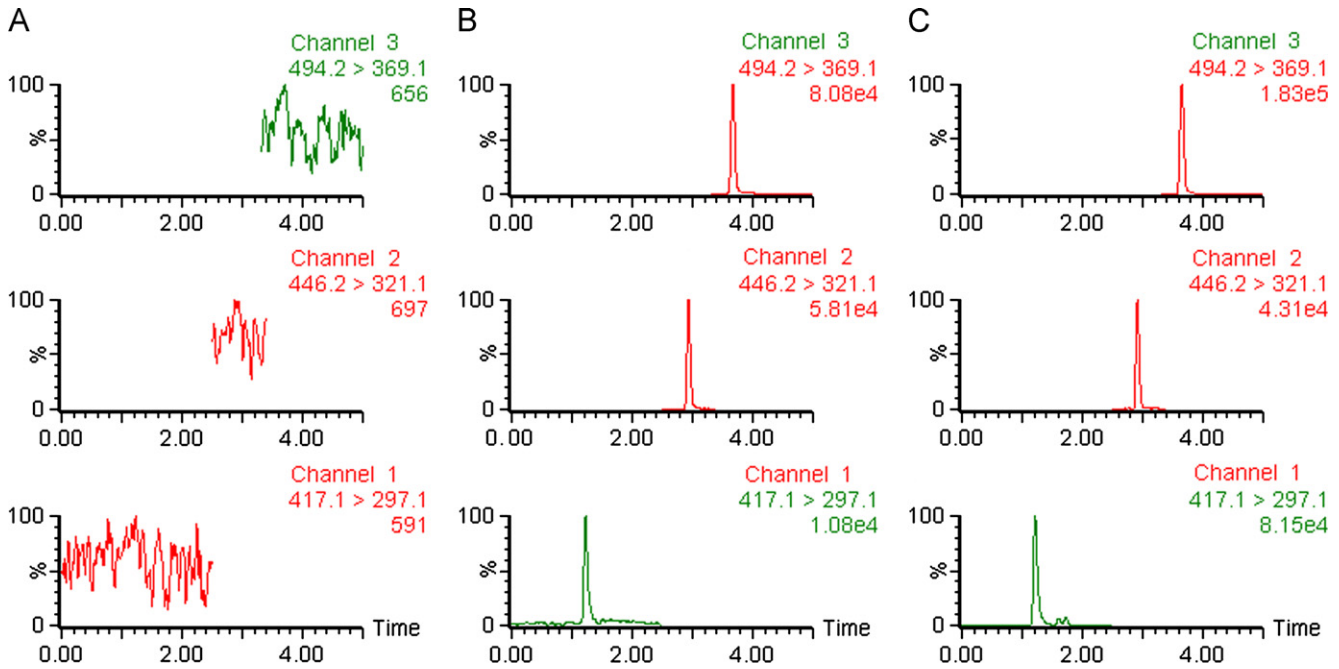

Fig. 2. Representative MRM chromatograms of puerarin (channel 1), glipizide (channel 2) and glibenclamide (channel 3) in rat plasma samples. (A) a blank plasma sample; (B) a blank plasma sample spiked with puerarin, glipizide and glibenclamide; (C) a plasma sample from a rat 0.5 h after an oral co-administration of *Pueraria lobata* extract and glibenclamide.

Table 1
Precision and accuracy for the determination of puerarin and glibenclamide in rat plasma (intra-run: $n=6$; inter-run: $n=6$ series per run, 3 runs).

Analyte	Added (ng/mL)	Found (ng/mL, mean ± S.D.)	Intra-run precision R.S.D. (%)	Inter-run precision R.S.D. (%)	Accuracy R.E. (%)
Puerarin	18.6	18.7 ± 1.7	7.0	19	0.76
	46.5	47.4 ± 3.3	6.0	12	1.9
	297.6	321.9 ± 25.4	7.5	10	8.2
	2976	3125 ± 157	5.2	2.9	5.0
Glibenclamide	4.28	4.27 ± 0.46	9.5	17	−0.23
	10.7	10.5 ± 0.7	5.6	13	−1.8
	68.48	72.31 ± 4.85	6.0	12	5.6
	684.8	628.4 ± 40.6	6.1	8.6	−8.2

Table 2
Stabilities of puerarin and glibenclamide in rat plasma samples.

Analyte	Nominal concentration (ng/mL; $n=5$)	Concentration found (ng/mL; mean ± S.D.)	R.S.D. (%)	R.E. (%)
Puerarin	Room temperature stability			
	46.5	45.1 ± 4.2	9.3	−3.0
	297.6	278.2 ± 27.0	9.7	−6.5
	2976	3129 ± 214	6.8	5.1
	Freeze-thaw stability			
	46.5	47.0 ± 3.8	8.1	1.1
	297.6	325.4 ± 15.9	4.9	9.3
	2976	2985 ± 68	2.3	0.32
	Post-preparative stability			
	46.5	49.9 ± 2.4	4.8	7.3
	297.6	312.6 ± 25.5	8.2	5.1
	2976	3129 ± 61	2.0	5.2
	Long-term stability			
	46.5	47.0 ± 6.0	13	1.1
	297.6	311.2 ± 16.2	5.2	4.6
	2976	3259 ± 143	4.4	9.5
Glibenclamide	Room temperature stability			
	10.7	10.0 ± 0.8	8.0	−6.5
	68.48	66.94 ± 2.84	4.2	−2.3
	684.8	626.4 ± 45.5	7.3	−8.5
	Freeze-thaw stability			
	10.7	10.7 ± 0.8	7.5	0
	68.48	65.99 ± 4.03	6.1	−3.6
	684.8	619.4 ± 26.1	4.2	−9.6
	Post-preparative stability			
	10.7	10.8 ± 0.7	6.5	0.93
	68.48	66.78 ± 6.93	10	−2.5
	684.8	602.4 ± 18.1	3.0	−12
	Long-term stability			
	10.7	10.2 ± 1.0	9.8	−4.7
	68.48	61.59 ± 3.37	5.5	−10
	684.8	594.8 ± 5.3	0.89	−13

the metabolism of glibenclamide. It was reported that glibenclamide was metabolized mainly in the liver by CYP 3A4 which was the most abundant hepatic and intestinal phase I enzyme that metabolized approximately 50% marketed drugs [30]. Thus, it was speculated that *P. lobata* extract inhibited CYP 3A4. For puerarin, increase in C_{max}, AUC and decrease in Cl/F and V_d/F were observed in presence of glibenclamide. These facts indicated that glibenclamide accelerated the absorption of puerarin. Previous study showed that glibenclamide could inhibit P-gp [31], it was therefore inferred that puerarin might be a substrate of P-gp and glibenclamide inhibited P-gp resulting in elevated puerarin concentration.

Given that a drug is highly bound by plasma proteins, it may displace the many herbal components from the binding sites. Therefore, the rat plasma protein binding rate of puerarin in the concentration range of 0.200–4.00 μg/mL was determined and it was 66.3 ± 4.9 (%). When with glibenclamide, it was reduced to 41.8 ± 4.0 (%). The results indicated that glibenclamide could compete for plasma protein binding with puerarin resulting in the increased free puerarin concentration. Subsequently, glibenclamide may improve the pharmacological effects of Chinese medicines containing puerarin, but may also increase the toxicity. The rat plasma protein binding rate of glibenclamide in the concentration range of 20.0–400 ng/mL was also determined and it was 99.4 ± 0.3 (%) and 99.2 ± 0.4 (%) without and with puerarin, respectively, indicating it was not disturbed by puerarin.

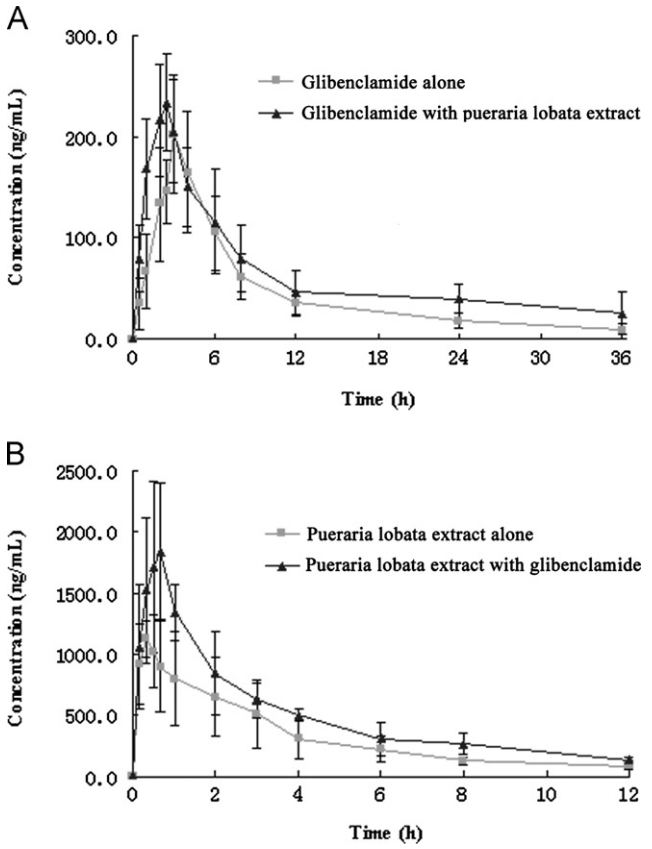

Fig. 3. The mean plasma concentration–time curves for glibenclamide and puerarin. (A) the curves for glibenclamide after oral administration of glibenclamide alone (10 mg/kg) (■) and co-administration with *Pueraria lobata* extract (615 mg/kg, equal to 400 mg/kg puerarin) (▲); (B) the curves for puerarin after oral administration of *Pueraria lobata* extract alone (615 mg/kg, equal to 400 mg/kg puerarin) (■) and co-administration with glibenclamide (10 mg/kg) (▲).

Table 3
Pharmacokinetic parameters of glibenclamide found in Group A, C's rats ($n=6$, mean ± S.D.).

Parameters	Group A[a]	Group C[b]
C_{max} (ng/mL)	214.0 ± 49.1	251.0 ± 43.2
T_{max} (h)	3.00 ± 0.63	2.42 ± 0.38
k_e (1/h)	0.063 ± 0.010	0.054 ± 0.010
$t_{1/2}$ (h)	11.20 ± 1.81	13.23 ± 2.41
AUC_{0-t} (ngh/mL)	1568 ± 470	2319 ± 637[c]
$AUC_{0-\infty}$ (ngh/mL)	1773 ± 522	2766 ± 779[c]
MRT_{0-t} (h)	9.03 ± 1.87	11.0 ± 1.0[c]
$MRT_{0-\infty}$ (h)	13.61 ± 2.40	17.75 ± 2.95[c]
Cl/F (L/(h · kg))	0.0060 ± 0.0016	0.0040 ± 0.0016[c]
V_d/F (L/kg)	0.096 ± 0.022	0.076 ± 0.031

[a] Group A: 10 mg/kg glibenclamide alone,
[b] Group C: 10 mg/kg glibenclamide co-administration with 615 mg/kg *Pueraria lobata* extract,
[c] $P<0.05$ compared to group A.

The pharmacokinetic interactions between drug and herbal medicines can significantly change the response of drug or herbal constituents, such as concentration or AUC. These changes can enhance drug efficacy, decrease toxicity or result in the converse outcome. For glibenclamide, hypoglycemicemia is a serious adverse effect which can lead to coma or death. It is suggested that the interaction of drugs with herbal medicines is a significant safety concern and needed to be investigated increasingly. This study was done with rats and not with humans, differences in the pharmacokinetic pattern between rats and human could exist. However, the results could provide some suggestion for clinical practice that the dosage should be adjusted or the drug concentration in plasma should be monitored if glibenclamide and puerarin were co-administered. We could also conclude that the interaction study with rats was of great importance and valuable reference.

Table 4
Pharmacokinetic parameters of puerarin found in Group B, C's rats ($n=6$, mean ± S.D.).

Parameters	Group B[a]	Group C[b]
C_{max} (ng/mL)	1259 ± 255	2003 ± 522[c]
T_{max} (h)	0.42 ± 0.18	0.56 ± 0.14
k_e (1/h)	0.155 ± 0.039	0.170 ± 0.030
$t_{1/2}$ (h)	4.73 ± 1.20	4.19 ± 0.72
AUC_{0-t} (ng h/mL)	3912 ± 1358	5976 ± 1098[c]
$AUC_{0-\infty}$ (ng h/mL)	4514 ± 1420	6822 ± 1116[c]
MRT_{0-t} (h)	3.45 ± 0.35	3.54 ± 0.28
$MRT_{0-\infty}$ (h)	5.62 ± 1.30	5.39 ± 0.62
Cl/F (L/(h kg))	0.095 ± 0.024	0.060 ± 0.011[c]
V_d/F (L/kg)	0.656 ± 0.243	0.359 ± 0.060[c]

[a] Group B: 615 mg/kg *Pueraria lobata* extract alone,
[b] Group C: 10 mg/kg glibenclamide co-administration with 615 mg/kg *Pueraria lobata* extract;
[c] $P<0.05$ compared to group B.

4. Conclusion

In the present study, a UPLC–MS/MS method for the simultaneous quantification of glibenclamide and puerarin in rat plasma has been developed and applied to study the pharmacokinetic interaction between them for the first time. The developed analysis method is simple, specific and reliable. The pharmacokinetic results showed that glibenclamide and puerarin were influenced mutually when being co-administered. The drug–herb interaction studies are of great importance for the sake of public health. More and systematic studies are needed to investigate the mechanism of interaction.

Acknowledgments

The study was supported by the Special Project of Shenyang Large Scientific Instrument Sharing Service (No. 2007GX-26).

References

[1] J. Xu, Y. Yang, Health Policy 90 (2009) 133–139.
[2] X.F. Ye, J. He, Contemp. Clin. Trials 31 (2010) 508–509.
[3] A.A. Izzo, E. Ernst, Drugs 61 (2001) 2163–2175.
[4] M.L. Chavez, M.A. Jordan, P.I. Chavez, Life Sci. 78 (2006) 2146–2157.
[5] S.F. Zhou, Z.W. Zhou, C.G. Li, X. Chen, X.Y. Yu, C.C. Xue, A. Herington, Drug Discovery Today 12 (2007) 664–673.
[6] W. Zhang, C.Q. Liu, P.W. Wang, S.Y. Sun, W.J. Su, H.J. Zhang, X.J. Li, S.Y. Yang, Eur. J. Pharmacol. 649 (2010) 398–402.
[7] K.H. Wong, G.Q. Li, K.M. Li, V. Razmovski-Naumovski, K. Chan, J. Ethnopharmacol. 134 (2011) 584–607.
[8] J.G. Shen, M.F. Yao, X.C. Chen, Y.F. Feng, Y.H. Ye, Z.H. Tong, Mol. Biol. Rep. 36 (2009) 2229–2233.
[9] J.Y. Yeo, Y.M. Kang, S.I. Cho, M.H. Jung, Chin. Med. 6 (2011) 1–10.
[10] H.M. Chiang, S.H. Fang, K.C. Wen, S.L. Hsiue, S.Y. Tsai, Y.C. Hou, Y.C. Chi, P.D.Lee Chao, Toxicol. Appl. Pharmacol. 209 (2005) 263–268.
[11] H.J. Chung, M.J. Chung, S.J. Houng, J. Jeun, D.K. Kweon, C.H. Choi, J.T. Park, K.H. Park, S.J. Lee, Eur. Food Res. Technol. 230 (2009) 145–153.
[12] S.M. Cui, Y.B. Ma, Zhongguo ZhongYao ZaZhi 33 (2008) 2033–2036.
[13] S.M. Cui, C.S. Zhao, J.J. Mao, Z.G. He, D.W. Chen, J. Shenyang Pharm. Univ. 21 (2004) 412–415.
[14] O.G. Kolterman, Clin. Ther. 14 (1992) 196–213.
[15] B.M. Rosenn, J. Matern, Fetal Neonatal Med. 23 (2010) 219–223.

[16] J.M. Feldman, Pharmacotherapy 5 (1985) 43–62.
[17] R. Böhm, Med. Monatsschr. Pharmazeuten 32 (2009) 453–458.
[18] J.J. Lilja, M. Niemi, H. Fredrikson, P.J. Neuvonen, Br. J. Clin. Pharmacol. 63 (2007) 732–740.
[19] P. Neerati, J. Gade, Eur. J. Pharm. Sci. 42 (2011) 285–289.
[20] Y. Ao, J. Chen, J. Yue, R.X. Peng, Eur. J. Pharmacol. 587 (2008) 330–335.
[21] N. Li, M. Cui, X.M. Lu, F. Qin, K. Jiang, F.M. Li, Biomed. Chromatogr. 24 (2010) 1255–1261.
[22] B.M. Chen, Y.Z. Liang, F.Q. Guo, L.F. Huang, F.L. Deng, X. Chen, Y.L. Wang, Anal. Chim. Acta 514 (2004) 185–191.
[23] F. Albu, C. Georgitã, V. David, A. Medvedovici, J. Chromatogr. B 846 (2007) 222–229.
[24] J.K. Prasain, N. Peng, E. Acosta, R. Moore, A. Arabshahi, E. Meezan, S. Barnes, J.M. Wyss, Biomed. Chromatogr. 21 (2007) 410–414.
[25] Q.Q. Wang, X.S. Li, S.J. Dai, L. Ou, X. Sun, B.Z. Zhu, F. Chen, M.M. Shang, H.F. Song, J. Chromatogr. B 863 (2008) 55–63.
[26] J.S. Markowitz, J.L. Donovan, C.L. DeVane, R.M. Taylor, Y. Ruan, J.S. Wang, K.D. Chavin, JAMA 290 (2003) 1500–1504.
[27] M. Modarai, J. Gertsch, A. Suter, M. Heinrich, A. Kortenkamp, J. Pharm. Pharmacol. 59 (2007) 567–573.
[28] C.W. Holtzman, B.S. Wiggins, S.A. Spinler, Pharmacotherapy 26 (2006) 1601–1607.
[29] D. Pal, A.K. Mitra, Life Sci. 78 (2006) 2131–2145.
[30] L. Zhou, S.B. Naraharisetti, L. Liu, H. Wang, Y.S. Lin, N. Isoherranen, J.D. Unadkat, M.F. Hebert, Q. Mao, Biopharm. Drug Dispos. 31 (2010) 228–242.
[31] P.E. Golstein, A. Boom, J. van Geffel, P. Jacobs, B. Masereel, R. Beauwens, Pflugers Arch. 5 (1999) 652–660.