

Pharmacokinetics and bioavailability study of polydatin in rat plasma by using a LC–MS/MS method

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Abstract: To investigate the pharmacokinetic and bioavailability of polydatin (PD) in rats after oral and intravenous administration, a simple, rapid and sensitive liquid chromatography-tandem mass spectroscopy (LC-MS/MS) method was developed and validated for the determination of polydatin. After precipitating the plasma proteins with methanol, the analytes were separated on a C₁₈ column (3.5 μ m, 2.1 \times 100 mm) with an isocratic mobile phase consisting of methanol-acetonitrile-0.1% formic acid (18: 15: 67, v/v/v) at a flow rate of 0.3mL/min. The Agilent G6410A triple quadrupole LC/MS system was operated under the multiple reaction monitoring (MRM) mode and the electrospray ionization technique was in negative mode. Linear responses were obtained for PD ranging from 1.0-5000.0 ng/mL ($r=0.9984$) and the LLOQ was 1.0ng/ml and was sufficient for the pharmacokinetic studies. The intra-day and inter-day accuracy and precision of the assay were less than 8.0%. The method is capable of quantifying PD. The pharmacokinetic parameters of polydatin after intragastric administration of PD with different doses (50, 100 and 300 mg/kg) and intravenous administration at the dose of 20 mg/kg, were obtained, with $t_{1/2}$ of 200.30 min, 210.30 min, 272.26 min, and 112.5 min, and AUC_{0- ∞} of 125626.41 μ g/L \cdot min, 250433.47 μ g/L \cdot min, 693722.60 μ g/L \cdot min and 1723509.57 μ g/L \cdot min, respectively. The absolute bioavailability of PD was somewhat low to 2.9%. The results were firstly reported, as far as we know, about bioavailability of PD and seem important for linking PD and other phenolic glycosides-related drugs administration to their medicinal effects.

Keywords: Polydatin; rat plasma; LC-MS/MS; pharmacokinetics; bioavailability.

INTRODUCTION

Polygonum cuspidatum Sieb. et Zucc is one of Traditional Chinese Medicine (TCM) in common use with its root and rhizome. The chemical constituents of *P. cuspidatum* have been studied and a variety of polyphenols have been isolated and identified in our previous research (Xiao *et al.*, 2000; Xiao *et al.*, 2002; Xiao *et al.*, 2002).

Polydatin (PD), 3, 4', 5-trihydroxystilbene-3-O- β -D-glucopyranoside, (shown in fig. 1) is a major effective chemical component with another name piceid, which is a glucoside of resveratrol (Kubo *et al.*, 1981). Previous studies have shown that polydatin had many pharmacological activities, such as protecting myocardial cells and enhancing heart function (Luo *et al.*, 1990; Miao *et al.*, 2011), preventing liver injuries (Kimura *et al.*, 1983), inhibiting the aggregation of platelets (Shan 1988), improving microcirculation (Zhu *et al.*, 1987) and lowering the serum levels of total cholesterol, triglyceride and low-density lipoprotein cholesterol (Xing *et al.*, 2009). Previous researches on PD have centered on the chemical extraction from herbs and pharmacological

effects. Little research involved the pharmacokinetics of PD and its analytical method in biological matrices (Gao *et al.*, 2006; Zhang *et al.* 2008; Cai Zhuo *et al.* 2009; Zhou *et al.* 2009).

Our previous study has established a RP-HPLC method for the determination of PD in rat plasma, bile, urine, feces and tissue homogenates after intravenous administration (Gao *et al.* 2006). The quantification limit of PD in rat plasma is 0.0251 μ g/g, The retention time for PD chromatogram is approximately 7.8 min. Zhang *et al.* reported a simultaneous determination of polydatin and its metabolite in excrement samples using gradient high-performance liquid chromatography (HPLC) with UV detection (Zhang *et al.*, 2008). The LLOQ of PD is 0.803 μ g \cdot ml⁻¹. The PD peak appeared at 14.5min. Cai *et al.* establish a method for detecting plasma PD using a HPLC method and investigate the pharmacokinetics of PD in Beagle dogs after intravenous administration. The LLOQ of PD is 0.1 μ g/ml (Cai Zhou *et al.*, 2009). These methods however required a long run time and were not so sensitive to meet the measurement of samples at low concentrations. Zhou *et al.* reported a liquid chromato-

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graphic-tandem mass spectrometric (LC-MS/MS) method for the quantification of PD in rat plasma that also need a long run time for about 10min with a complex and time-consuming sample pretreatment (Zhou *et al.*, 2009). Zhou *et al.* also reported dose-dependent absorption and metabolism of PD in rats after an oral administration of PD at three different doses (Zhou *et al.*, 2009). However, the bioavailability of PD still has not been studied and evaluated.

In this study, more attention has been paid to develop and validate a simple, sufficiently sensitive, and rapid LC-MS/MS method for the quantitative analysis of PD in rat plasma. The method was capable of quantifying PD at a low concentration of 1.0ng/ml and would meet the analysis need of plasma samples from pharmacokinetic studies. The pharmacokinetics and bioavailability of PD in rats after the oral administration of PD at three different doses were also investigated.

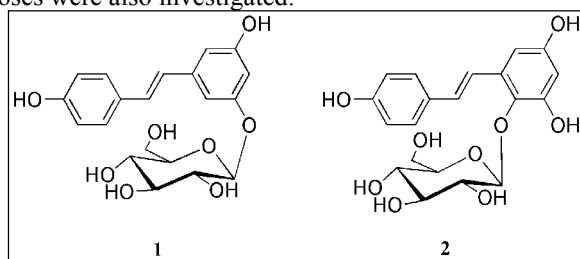


Fig. 1: Chemical structures of PD (1) and TSG (2)

MATERIALS AND METHODS

Materials

PD and 2,3,4,5-tetrahydroxystilbene-2-O- β -D-glucopyranoside (TSG, IS, >99% purity) were obtained from National Institute for the Control of Pharmaceuticals and Biological Products of China (Beijing, China). PD injections and standard reference material were provided by Haiwang Pharmaceuticals Co. Ltd. (Guangdong, China). HPLC grade methanol and acetonitrile were obtained from Merck Company (Darmstadt, Germany). Formic acid was purchased from TEDIA Company (Tedia Fairfield, OH, USA). Ultrapure water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA). All other reagents were of analytical grade.

Instruments

The experiments were carried out on an Agilent 1200 series HPLC system which interfaced to an Agilent 6410A triple-quadrupole mass spectrometer. The mass spectrometer was equipped with an electrospray ionization source (Agilent Inc, MA, USA). The Agilent 6410A triple quadrupole LC-MS system equipped with G1311A quaternary pump, G1322A vacuum degasser, G1329A autosampler and G1316A therm. Column compartments was used for all analyses. MassHunter software (Agilent Inc, MA, USA) was applied to control the whole system.

Chromatographic conditions

A ZORBAX SB-C18 (3.5 μ m, 2.1 \times 100 mm, Agilent Corporation, MA, USA) was used as the analytical column. The mixture of methanol-acetonitrile-0.1% formic acid (18: 15: 67, v/v/v) served the mobile phase. By the electronic degasser system, the mobile phase could degasse automatically. The column was equilibrated and eluted under isocratic conditions. The flow rate of the column was 0.3mL/min. The column temperature was 30°C and the injection volume was 10 μ L.

Mass spectrometric conditions

Electrospray ionization technique was in the negative mode and spray voltage was set at 4000 V. The nebulizer gas was Nitrogen. The pressure of the nebulizer was 40 psi and the source temperature was 105°C. Heat desolvation gas (nitrogen) to 350°C, then deliver it at a flow rate of 10 L/min. For collision-induced dissociation (CID), high purity nitrogen was used as collision gas at a pressure of 0.1 MPa. Multiple reaction monitoring (MRM) mode at m/z 389.3 \rightarrow 227.1 for PD and m/z 405.1 \rightarrow 243.2 for IS were applied for quantification. The fragmentor energies of MS¹ for PD and IS were set at 180 and 175 V. The optimized collision energies of 15 and 10 eV were used for PD and IS. The peak widths of precursor and product ions were maintained at 0.7 amu at half-height in the MRM mode.

Preparation of standard and quality control (QC) samples

The standard stock solutions of PD was prepared in methanol to final concentrations of 1.0 mg/mL. Pooled rat plasma containing no PD was used as the blank plasma to prepare calibration standards and QC samples for this study. The calibration standards of PD at concentrations of 1.0, 2.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0, 1000.0, 2000.0 and 5000.0 ng/mL were prepared by spiking appropriate amounts of the standard solutions in blank plasma. The stock solution of IS was 100 μ g/mL and was prepared by dissolving the drug in methanol. This solution was diluted to a final concentration of 100 ng/mL by water. All the solutions were stored at 4°C and brought to room temperature before using them.

Quality control samples for PD was made up in plasma by an independent analyst using a new stock solution, representing low, medium and high concentration of QC samples respectively. Samples were aliquoted into cryovials, and stored frozen at -20°C for use with each analytical run. Accepting or rejecting the run based on the results of the QC samples.

Sample preparation

By adding 300 μ L of methanol containing the IS (100 ng/mL), a 100 μ L aliquot of plasma sample was prepared. The mixture was then vortex-mixed for 1 min and centrifuged at 2000 \times g for 10 min, the supernatant was added to 200 μ L mobile phase, vortex-mixed for 1 min

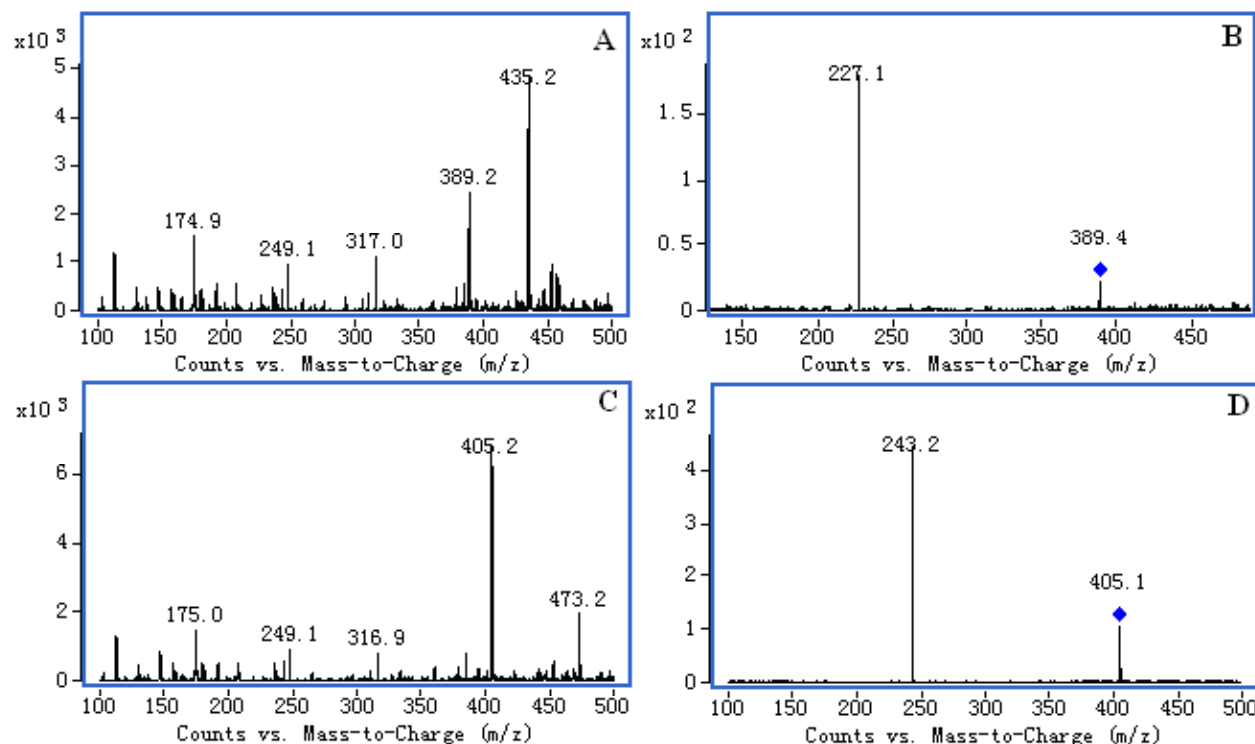


Fig. 2: ESI-MS spectrum of polydatin and IS. (A) full-scan of polydatin, (B) product ion of polydatin, (C) full-scan of IS, (D) product ion of IS

and centrifuged at $2000\times g$ for 5 min. Then a $10\mu\text{L}$ aliquot of supernatant was injected into the LC-MS/MS system.

Assay validation

The standard curves ranging from 1.0 to 5000.0 ng/mL PD were obtained after running the column on three separate days. Calibration curves were constructed from the peak area ratios of each analyte to IS versus plasma concentrations using a $1/x^2$ weighted linear least squares regression model.

To determine the intra-day and inter-day precision of the assay, six replicates of QC samples at three levels of PD were included in each run. The accuracy was gotten by the percentage difference between the mean detected concentrations and the nominal concentrations. The lowest concentration of standard which could be measured with an acceptable accuracy and precision ($\leq 15\%$ for both parameters) is defined as the lower limit of quantification (LLOQ).

The extraction recoveries of the three analytes at high, middle and low QC levels were determined by comparing peak areas obtained from plasma samples with those found by direct injection of a standard solution of the same concentration ($n=5$ each).

By analyzing triplicate QC samples stored for 6 h at ambient temperatures, three cycles of freezing at -20°C

and thawing, reconstituted extract at room temperature for 24 h and stored for 1 month at -20°C , respectively, the stability of three analytes in plasma was assessed. Concentrations following storage were compared with freshly prepared samples of the same concentrations.

Application of the analytical method

Twenty-four Male Sprague-Dawley rats, weighing approximately 230-250 g, were obtained from Shanghai SLAC Lab Animal Co., Ltd. (Shanghai, China). The animal experimentation was approved by the Second Military Medical University Animal Ethics Committee (Shanghai, China). Eighteen rats received intragastric administration of PD in water/carboxymethylcellulose sodium (CMC-Na) (0.5% w/v) with different doses (50, 100 and 300 mg/kg). At 0, 10, 20, 30, 60, 90, 120, 180, 240, 360, 480, 720, 960 and 1440 min after administration, rat blood samples ($250\mu\text{L}$) were collected in heparinized tubes. Six rats received PD intravenously at the dose of 20 mg/kg. Blood samples ($250\mu\text{L}$) were collected before the administration and at post-dose at 5, 10, 20, 40, 60, 90, 120, 180, 240, 300, 360, 480 min, respectively. The blood samples were placed in heparinized tubes and immediately centrifuged and then stored at -20°C until analysis.

All pharmacokinetic parameters were determined by non-compartmental analysis. The peak plasma level (C_{max}) and the time to reach the peak plasma concentration (t_{max})

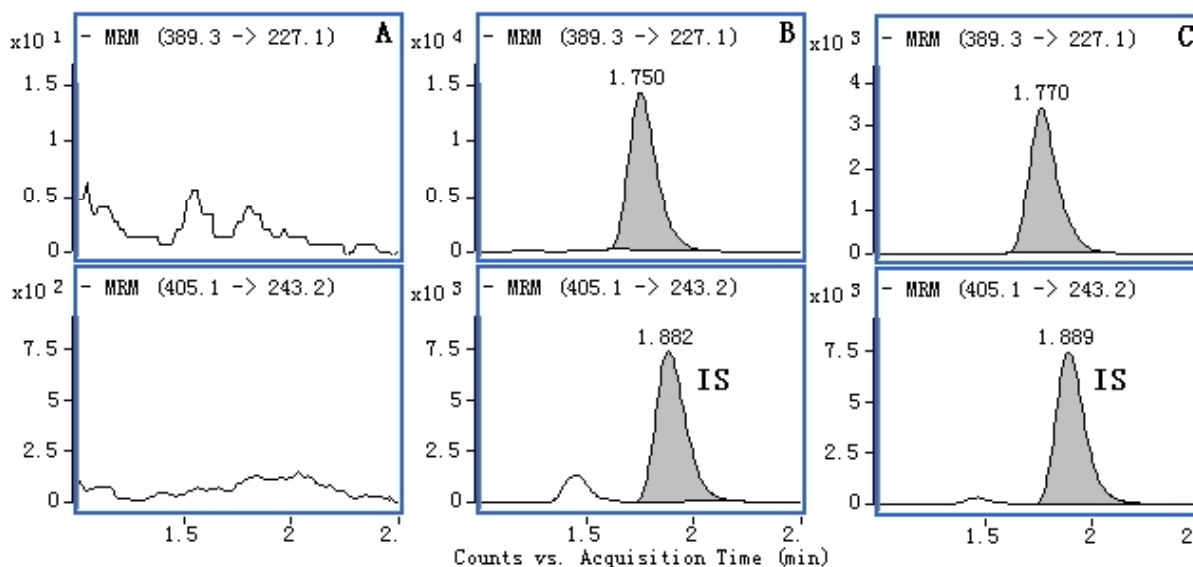


Fig. 3: Representative MRM chromatograms of polydatin plasma samples. (A) Blank plasma, (B) test plasma sample, (C) blank plasma sample spiked with polydatin

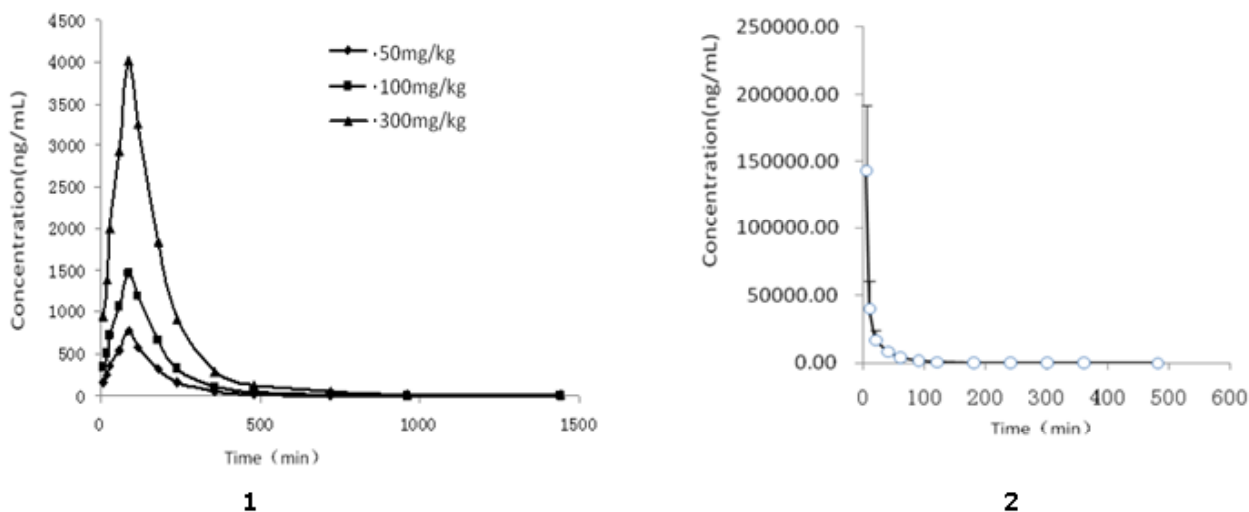


Fig. 4: Mean plasma concentration-time profile of polydatin in rats after oral administration of 50, 100 and 300 mg/kg (1) and intravenous administration of 20 mg/kg (2)

were obtained directly from the concentration-time data. The elimination rate constant (K_e) was calculated from the slope of the logarithm of the plasma concentration versus time using the final four points. The apparent elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule. Each value was expressed as mean \pm S.D. Statistical significance was determined using Student's t-test and one-way analysis of variance (ANOVA), $P < 0.05$ was regarded as significant difference.

RESULTS

LC-MS/MS optimization

PD and IS were at first characterized by MS^2 scan and MS-MS product ions to ascertain their precursor ions and to select product ions for use in MRM mode, respectively. To get the richest relative abundance of precursor ions and product ions, the parameters for fragmentor energies and collision energies were optimized, and the MRM transition were chosen to be m/z 389.3 \rightarrow 227.1 for PD and m/z 405.1 \rightarrow 243.2 for IS. The spectra of full scan production of precursor ions of the PD and IS were showed in fig.2.

Table 1: Intra-day and inter-day precision and accuracy of PD in rats plasma (n=5)

Concentration added (ng/mL)	Intra-day			Inter-day		
	Concentration found (mean \pm SD, ng/mL)	Precision RSD %	Accuracy RME %	Concentration found (mean \pm SD, ng/mL)	Precision RSD %	Accuracy RME %
20.0	19.98 \pm 0.73	3.71	-0.12	19.90 \pm 1.56	7.92	-0.57
200.0	199.53 \pm 7.87	4.03	-0.34	196.17 \pm 10.93	5.61	-2.02
2000.0	1979.08 \pm 47.96	2.45	-1.11	1975.20 \pm 42.60	2.26	-1.31

RME is expressed as (mean measured concentration)/(spiked concentration)-1 \times 100%

Table 2: Mean pharmacokinetic parameters of PD after oral administration of PD at the dose of 50, 100 and 300 mg/kg and intravenous administration at the dose of 20 mg/kg (n=6)

Parameter	Unit	Intravenous Dose (20 mg/kg)	Oral Dose (mg/kg)		
			50	100	300
T _{max}	min	5.00	90	90	90
C _{max}	μ g/L	143170.93	777.55	1461.91	4016.01
C _{max} /dose		7158.55	15.55	14.62	13.39
T _{1/2z}	min	112.5	200.30	210.30	272.26
MRT	min	31.19	170.33	171.85	172.52
AUC ₍₀₋₁₄₄₀₎	μ g/L*min	1712407.66	125320.47	249821.58	690344.38
AUC _(0-∞)	μ g/L*min	1723509.57	125626.41	250433.47	693722.60
Bioavailability (%)		/	2.93	2.92	2.69

Evaluation of mobile phases containing various combinations of methanol, acetonitrile, 0.1% formic acid and water showed that inclusion of acetonitrile produced strong signals with no solvent-clustered ions. The inclusion of 0.1% formic acid made the efficiency of ionization better and gave satisfactory sensitivity. With the presence of formic acid in the mobile phase, the peak shapes were improved and matrix effects were reduced, and there were no significant decrease in response.

Method Validation

Assay selectivity and matrix effect

Because of only monitoring the selected ions produced from selected precursor ions, the LC-MS/MS method had high selectivity (shown in fig. 2). Comparison of the chromatograms of the blank and the spiked rat plasma (shown in fig.3) indicated no significant interference at the retention times of the analytes and the IS. The results of matrix effect experiments showed that there was no significant difference between the peak areas of samples prepared from rat plasma and from water. The results also indicated that no co-eluting unseen compounds significantly influenced the ionization of analytes and IS.

Linearity and lower limit of quantification (LLOQ)

Linear responses were obtained for PD ranging from 1.0 to 5000.0 ng/mL. A typical calibration equation was $Y=0.0023C + 0.0892$ ($r=0.9984$), where Y represents the peak area ratios of PD to the IS and C represents the plasma concentrations of PD. The present method offered a LLOQ of 1.0 ng/mL with R.S.D. of 13.1%. The LLOQ was sufficient for the pharmacokinetic studies of PD in rats.

Assay precision and accuracy

The method reviewed the good precision and accuracy. Intra-day and inter-day precision and accuracy for PD from plasma samples data were shown in table 1. The intra-day and inter-day precisions were ranging 2.5-4.0% and 2.2-7.9% for PD, respectively. These results suggested that the procedures described as above were satisfactory with respect to both accuracy and precision. All intra-day and inter-day precision and accuracy were acceptable. Analytical values of all QC samples were all within the 10% of their respective nominal values.

Extraction recovery

Mean extraction recoveries of PD under the protein precipitation conditions were 79.3%, 80.2%, 78.5% for 20.0ng/mL, 200.0ng/mL and 2000.0ng/mL, respectively, and their CV values were 4.4%, 6.3% and 3.3%, respectively.

Analyte stability

The stability of PD in rat plasma and mobile phase were both investigated. The analyte was found to be stable in rat plasma stored for 1 month at -20°C and in reconstituted mobile phase at room temperature for 24 h (<5% reduction). After storage at 1-4°C for 2 month, there was no obvious reduction in the stock and working solutions. After three freeze-thaw cycles, the reduction was less than 10% indicating that the analyte was stable. The analytes were also shown to be stable in rat plasma at room temperature for at least 6 h with a reduction of less than 10%.

Application of the analytical method to pharmacokinetic and bioavailability studies

The LC-MS/MS method developed was used to investigate the pharmacokinetics of PD after oral administration 50, 100 and 300 mg/kg of PD solution to eighteen rats and intravenous administration 20 mg/kg to six rats, respectively. Table 2 shows the main pharmacokinetic parameters of PD and fig. 4 illustrates the mean plasma concentration-time profiles of PD after oral and intravenous administration of PD, respectively.

CONCLUSION

In this paper, ESI negative ion mode was adopted to optimize the ionization condition of PD and its internal standard TSG. For MS/MS determination, [M-H]⁻ peak could easily generate regular fragments. Moreover, the optimized condition of ionization and fragmentation coupled with the improved high-energy dynode voltages can finally satisfy the determination of the plasma concentration of PD and TSG.

The pharmacokinetic parameters of polydatin after intragastric administration of PD with different doses (50, 100 and 300 mg/kg) and intravenous administration at the dose of 20 mg/kg, were obtained, with a terminal half-life ($t_{1/2}$) of 200.30 min, 210.30 min, 272.26 min, and 112.5 min and AUC_(0-∞) of 125626.41 μg/L·min, 250433.47 μg/L·min, 693722.60 μg/L·min and 1723509.57 μg/L·min, respectively. Intravenous administration resulted in a much higher increase in peak plasma concentrations and a more rapid elimination as compared to the oral dose. When the oral dose increased from 50 to 300 mg kg⁻¹, the C_{max} and AUC_{0-∞} increased linearly in a dose-dependent manner ($r=0.9999$ and 0.9998 , respectively), while the $t_{1/2}$ were similar ($P>0.05$) and showed dose independence. Although the $t_{1/2}$ of oral administration were a little higher than that of intravenous administration, there is no significance between the four groups ($P>0.05$). These results were consistent with the previous study (Zhou *et al.*, 2009).

DISCUSSION

At the beginning of the study, the protein precipitant had been explored and the results indicated that the extraction recovery was higher with methanol. The peak shape and response would be particularly unacceptable and low when the supernatant was directly injected into the LC-MS. After diluting the supernatant with water, mobile phase, 0.1% formic acid, 0.3% formic acid, 0.5% formic acid in the ratio of 1: 1 and 1: 2, respectively, the results showed that the supernatant diluted with the mobile phase in 1: 1 ratio had the highest recovery (almost 78%). This method requires less plasma samples and has high selectivity and sensitivity with reduced analysis time compared with the literature (Zhou S, *et al.* 2009). The LLOQ is 1.0ng/mL and there is no interference of

impurities in the blank plasma. Overall, this method could completely meet the pharmacokinetic research requirement of PD with the low concentration after an intragastric administration.

The absolute bioavailability of PD was somewhat low (2.9%) possibly because of glycoside's unfavorable physicochemical traits, such as high hydrophilicity (Yu *et al.*, 2012). These results were firstly reported, as far as we know, about bioavailability of PD and seem important for linking PD and other phenolic glycosides-related drugs administration to their medicinal effects.

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