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ORIGINAL ARTICLE

Pharmacokinetic evaluation of paeoniflorin after oral administration of Paeoniae Radix extract powder to healthy Korean subjects using UPLC-MS/MS

Seo-Hee Heo[1] · Dong-Seok Lee[1] · Seong-Ho Ham[2] · Jung-Hee Cho[2] ·
Young-Dal Kwon[3] · Yong-Bok Lee[4] · Hea-Young Cho[1]

Abstract Paeoniae Radix, a traditional herbal medicine, has been used in antispasmodic action, alleviating pain, and activating circulation. The aim of this study was to evaluate the pharmacokinetics (PKs) of paeoniflorin in healthy Korean subjects and develop an analytical method for the quantification of paeoniflorin in human plasma. This was an open-label, randomized, and single-dose study in 10 healthy Korean subjects who received a Paeoniae Radix extract powder. The plasma concentration of paeoniflorin up to 12 h was determined using a validated ultra-performance liquid chromatography tandem mass spectrometric method. The PK parameters such as $AUC_{0-\infty}$, CL/F, V_d/F, C_{max}, T_{max} and $t_{1/2}$ were calculated using WinNonlin® software (version 6.4, Pharsight®, a Certara™ Company). The best PK model of paeoniflorin in humans was the one-compartment model. The mean parameters were 2625.71 L/h, 10,150.55 L, 6.97 ng/mL, and 1.64 h for the oral clearance (CL/F), the volume of distribution (V_d/F), the maximum plasma concentration (C_{max}), and the time to reach C_{max}(T_{max}), respectively. The elimination half-life

($t_{1/2}$) was 2.68 h. The validated method was successfully applied to the PK study of paeoniflorin in humans. The lower limit of quantification for paeoniflorin in human plasma was 0.2 ng/mL. This study was the first to evaluate the PKs of paeoniflorin after the usual oral dose of Paeoniae Radix extract powder (3.4 g containing 108.18 mg paeoniflorin) in Korean subjects.

Keywords Paeoniflorin · Pharmacokinetics · Human · UPLC-MS/MS

Introduction

Traditional herbal medicine is increasingly important around the world (Ahmad et al. 2015). Traditional Korean medicine generally includes many herbs, which also comprise various components. They were widely known to treat and prevent many diseases associated with multiple targets (Qiu 2007; Wang et al. 2008). Paeoniae Radix, one of the well-known medical herbs in many countries, is the root of *Paeonia lactiflora Pallas* (Yamahara et al. 1982; Zhang et al. 2012). In general, it has been used in nourishing blood, activating circulation, alleviating pain, regulating menstruation, and treating liver disease and cancer (Lee et al. 2002). Also, Paeoniae Radix might inhibit the growth of hepatoma tumor cells (Lee et al. 2002). In Korea, the 1.36–3.40 g of Paeoniae Radix extract powder is orally administered three times daily in patients with pain and convulsion according to the approval by Ministry of Food and Drug Safety.

Paeoniflorin, a monoterpene glycoside ($C_{23}H_{28}O_{11}$), is the main and active component of Paeoniae Radix (Yamahara et al. 1982) with various biological activities such as anti-inflammatory, anti-oxidative, and immune-

✉ Hea-Young Cho
hycho@cha.ac.kr

[1] College of Pharmacy, CHA University, 335 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13488, Republic of Korea

[2] Jeonnam Development Institute for Korean Traditional Medicine, 288 Udeuraendeu-gil, Jangheung-gun, Jeollanam-do 59338, Republic of Korea

[3] Department of Oriental Rehabilitation Medicine, Wonkwang University Hospital, 1140-23 Hoejae-ro, Nam-gu, Gwangju 61729, Republic of Korea

[4] College of Pharmacy and Institute of Bioequivalence and Bridging Study, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 61186, Republic of Korea

regulatory activities (Yamahara et al. 1982; Jiang et al. 2009; Zhang et al. 2012; Chen et al. 2013). These biological activities are significantly related to the down-regulation of inflammatory infiltration and prevent alveolar bone loss and soft-tissue destruction. These characteristics suggest that it may be a potential anti-inflammatory agent for periodontitis (Ni et al. 2016). Because paeoniflorin attenuates cognitive impairment, improves hippocampus perfusion, and inhibits inflammatory response in vascular dementia rats, it may be of potential use for the treatment of vascular dementia (Zhang et al. 2015a). Moreover, it might be of a potential clinical application in improving neuropathic pain and associated insomnia owing to its analgesic and hypnotic effects via adenosine A_1 receptor (Yin et al. 2016).

In the pharmacokinetic (PK) studies, the bioavailability of paeoniflorin is low (3–4 %) when orally administered, because of the limited transportation of paeoniflorin across the gastrointestinal mucosa (Takeda et al. 1995, 1997). The mean $t_{1/2}$ of paeoniflorin has been reported as 55.70 min (Li et al. 2008), 2.4 h (Li et al. 2014) and 1.73–1.74 h (Sadakane et al. 2015) in humans. The absorbed paeoniflorin was mainly excreted in urine, because approximately 50 % of the dose was found in urine at 30 min after the intravenous administration, with little being excreted in the feces and bile (Takeda et al. 1995).

During the past decade, the analytical methods using high-performance liquid chromatography (HPLC) in rat (Liu et al. 2005) and humans (Li et al. 2008) have been used to evaluate the PKs of paeoniflorin. Several studies applying liquid chromatography tandem mass spectrometry (LC-MS/MS) method have been reported for the quantification of paeoniflorin in rats (Tong et al. 2010; Jiang et al. 2012; Li et al. 2013; Wang et al. 2013a, b; Chen et al. 2014; Yan et al. 2014; Ai et al. 2015; Hu et al. 2015; Zhang et al. 2015b; Zhao et al. 2015) and humans (Li et al. 2014; Sadakane et al. 2015).

The PK studies of paeoniflorin have been reported in healthy Japanese and Chinese male subjects (Li et al. 2008, 2014; Sadakane et al. 2015), but studies involving Korean subjects for paeoniflorin havenot been published. In general, the results of PK study are affected by the ethnicity, and dosage sometimes needs to be adjusted for different ethnic groups. Moreover, confirming the similarity of the PKs for paeoniflorin in Asian subjects is necessary. Therefore, the aim of this study was to evaluate the PKs of paeoniflorin after the usual oral dose administration of Paeoniae Radix extract powder (3.4 g containing 108.18 mg paeoniflorin) in Korean subjects and compare the PK parameters of paeoniflorin with Asian subjects. In addition, a more sensitive analytical method for the quantification of paeoniflorin in human plasma was developed.

Materials and methods

Materials

Paeonia lactiflora Pallas root extract powder (Paeoniae Radix extract powder), which is dried powder after extraction by water from Paeoniae Radix, was produced in Hanzung Pharmaceutical Co., Ltd. (Daejeon, Korea). Paeoniflorin (purity >98 %) and geniposide (internal standard, IS) were supplied from Sigma-Aldrich (St Louis, MO, USA), and their structures are shown in Fig. 1. Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Formic acid was supplied by Sigma-Aldrich (St Louis, MO, USA). The other chemicals were of HPLC grade or highest quality available. HPLC grade water (18.2 MΩ)was obtained from an Elga Purelab Option-Q system (Elga LabWater, Marlow, UK) and used throughout this study.

Content of paeoniflorin

Paeoniae Radix extract powder (0.5032 g) was accurately weighed and sonicated in 25 mL of 50 % methanol/water for 30 min and then filtered. 25 mL of 50 % methanol/
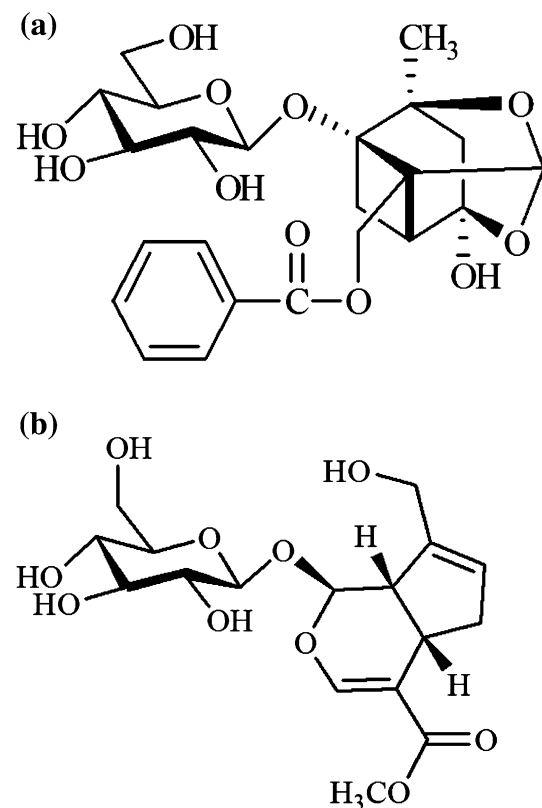

Fig. 1 Chemical structure of **a** paeoniflorin and **b** geniposide (IS)

water was added to extract the residual extract powder and under a reflux condenser for 1 h. After repeated filtrations and vacuum evaporation, 50 % methanol was added to the residue to make the volume exactly 50 mL. This extracted solution was filtered using a 0.45 μm syringe filter. Separately, 1 mg of paeoniflorin reference standard (RS) was weighed and dissolved in 1 mL of methanol to make at 1 mg/mL. The RS was diluted with 50 % methanol/water to achieve the final concentrations of 100 μg/mL, and the resulting solution was used as the standard solution. Each 20 μL of the analyte and the standard solution were injected directly into HPLC (Alliance® HPLC e2695 system with 2489 UV/Vis Detector, Waters Corp., Milford, MA, USA). The paeoniflorin content was analyzed by HPLC using Waters Nova-Pak® C_{18} (3.9 mm × 150 mm, 4 μm particle size, Waters, Milford, MA, U.S.A.) column. The mobile phase was water and acetonitrile using gradient elution at a flow rate of 1.0 mL/min at 254 nm. The peak areas of paeoniflorin of the test solution (A_T) and the standard solution (A_S) were determined by the following equation.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$)

$$= \text{amount (mg) of paeoniflorin RS} \times \frac{A_T}{A_S}$$

Subjects

The PK study was conducted as open-label, randomized, and single-dose study at the Wonkwang Oriental Medicine Hospital, Gwangju, Korea. Ten normal healthy male Korean subjects (20–30 years, 58.7–82.7 kg, 162.7–183.0 cm) took part in this study of oral Paeoniae Radix extract powder after providing written informed consent. The study protocol was approved by the Institutional Review Board (IRB) of the Wonkwang Oriental Medicine Hospital, Gwangju, Korea (https://cris.nih.go.kr No. KCT0001118). This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practice. The medical history of each subject was reviewed, and the absence of alcohol- and/or drug-abuse problems was a prerequisite. The general physical examination and laboratory tests were conducted. All the subjects fasted for at least 10 h before the administration of the drug and continued to fast for 4 h thereafter. They were abstained from the consumption of xanthine-containing foods and beverages during the study. Each subject received the usual oral dose of Paeoniae Radix extract powder (3.4 g) with 240 mL of spring water.

Blood samples were drawn from the forearm vein at 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 h after the oral drug administration. The samples were collected in Vacutainer® (10 mL, Becton–Dickinson and Company, USA) tubes and immediately centrifuged (10,000×g, 10 min, 4 °C) to obtain plasma sample. Following centrifugation, 5 mL of plasma sample was transferred to a polyethylene tube and stored at 70 °C until analysis.

Pharmacokinetic study

The PK parameters were calculated by non-compartmental and compartmental analysis of the plasma concentration–time curves of paeoniflorin using WinNonlin® software (version 6.4, Pharsight®, a Certara™ Company). The area under the curve ($AUC_{0-\infty}$) was calculated by the trapezoidal method to the last measurable concentration and extrapolated to infinity by using the equation $AUC_t + C_t/k$ (k was the terminal rate constant). The clearance (CL/F) was determined as the dose of paeoniflorin divided by $AUC_{0-\infty}$, and the volume of distribution (V_d/F) was calculated as clearance divided by the k, where F is the oral bioavailability. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained by visual observation of the individual plasma concentration–time profiles for paeoniflorin. The elimination half-life ($t_{1/2}$) was calculated as 0.693/k. The values of all the PK parameters were expressed as the estimate value ± standard deviation (mean ± S.D., n = 10).

Instruments and chromatographic conditions

The UPLC system consisted of Agilent (Agilent 1260 Series, Agilent Technologies, Palo Alto, CA, USA), and a triple quadrupole Agilent 6495 mass spectrometer with an electrospray ionization (ESI) source from Agilent Technologies (Palo Alto, CA, USA) and used for the detection of paeoniflorin. Different mobile phases were tested using a Phenomen ex Kinetex Biphenyl 100A column (2.1 mm × 50 mm, 1.7 μm particle size, Phenomenex, Torrance, CA, USA) with a column temperature of 40 ± 5 °C for the separation of the permanently charged analyte. 0.1 % formic acid in water as mobile phase A and acetonitrile as mobile phase B was used at a flow rate of 0.3 mL/min. The gradient elution program was as follows: the initial mobile phase was maintained at 10 % (B) for 1.5 min, linearly increased to 30 % (B) up to 3.0 min, then linearly increased to 90 % (B) over 3.0 min and held constant for up to 1.3 min, and finally returned to the initial condition (1.5 min). The mass spectrometer was operated using the ESI interface in the negative ion mode. Multiple reaction monitoring (MRM) transitions, m/z 525.2 → 121.0 and m/z 433.1 → 225.1 for paeoniflorin and IS, respectively, were used for quantification. The following parameters were used: capillary voltage, 2.8 kV; ion source

temperature, 150 °C; and desolvation temperature, 350 °C. Nitrogen was used as the cone and desolvation gas at a flow rate of 150 and 550 L/h, respectively. The optimized collision energy was13 eV for paeoniflorin and IS. The cone voltage of paeoniflorin and IS were 23 V and 22 V, respectively. Argon gas was used at a pressure of $\sim 4.2 \times 10^{-3}$ mbar. Data acquisition and analysis were achieved using an Agilent masshunter (Agilent Technologies, Palo Alto, CA, USA).

Preparation of calibration standards and quality control (QC) samples

The stock solutions of paeoniflorin and IS were prepared in 100 % methanol at the concentrations of 1 mg/mL and stored at −20 °C until dilution. The standard curve for paeoniflorin in human plasma ranged from 0.2 to 20 ng/mL. To prepare a calibration curve of 0.2, 0.5, 1, 2, 5, 10, and 20 ng/mL, blank plasma was spiked with the working solutions of paeoniflorin. In addition, the QC samples for paeoniflorin were prepared in blank plasma at the concentrations of 0.6 ng/mL (low), 3.2 ng/mL (medium) and 16 ng/mL (high) in the same manner as the calibration curves. The calibration and QC samples were freshly prepared on the day of analysis. A working solution for IS (100 ng/mL) was also prepared using same diluent.

Sample preparation

200μL of human plasma was spiked with 10 μL of IS (100 ng/mL in 50 % methanol) solution. Then, 800 μL of 100 % methanol was added, vortex-mixed for 3 min, and centrifuged at $10,000 \times g$ for 5 min at room temperature. 800 μL of supernatant was transferred to clean tubes and evaporated to dryness under a gentle nitrogen stream at 50 °C. The extraction residue was reconstituted in 75 μL of 20 % methanol in the presence of 0.1 % formic acid and vortex-mixed for 1 min. After 5 min of centrifugation at $10,000 \times g$, 5 μL aliquots were injected into the UPLC-MS/MS system.

Method validation

The analytical method was validated for selectivity, sensitivity, linearity, accuracy, precision, recovery and stability according to the principles of the Guidance for Industry: Bioanalytical Method Validation established by FDA (US Food and Drug Administration 2013).

Specificity

The specificity of the method was determined by evaluating the interferences of the endogenous compounds using screening analysis of drug-free plasma, plasma spiked with paeoniflorin, and plasma samples obtained after the usual oral dose administration with Paeoniae Radix extract powder (3.4 g) to Korean subjects.

Linearity and lower limit of quantification (LLOQ)

The linearity of calibration curves constructed by weighted (1/concentration^2) linear least-squares regression was determined by plotting the peak area ratios of paeoniflorin to the IS versus the nominal concentration. Linearity was tested with the paeoniflorin concentrations in the range from 0.2 to 20 ng/mL. A straight-line regression equation was obtained with a correlation coefficient (r^2) of 0.99.

The sensitivity of the method was expressed as the LLOQ determined as the lowest concentration of the standard samples with a signal-to-noise ratio of at least 10:1, in accordance with an acceptable precision of less than 20 % and accuracy within ± 20 %, which were evaluated using five replicate samples.

Precision and accuracy

Intra-day precision and accuracy were determined by the analysis of the spiked paeoniflorin plasma samples at the calibration concentrations and the QC samples (LLOQ = 0.2 ng/mL, low concentration = 0.6 ng/mL, medium concentration = 3.2 ng/mL and high concentration = 16 ng/mL) at five different times on the same day. Inter-day assessments were similarly carried out on five consecutive days. The concentration of each QC sample was evaluated using freshly prepared calibration standards. The precision was determined by calculating the coefficient of variation (CV) for the analysis of the QC samples of five replicates on the same day and five consecutive days. The CV of precision for each concentration level should not deviate by more than ±15 % except for the LLOQ with a limit of 20 %.The accuracy was evaluated based on the following criteria: the mean value should not exceed 15 % of the nominal concentration except for the LLOQ, which should not exceed 20 %.

Recovery

The recovery of paeoniflorin was evaluated for the QC samples at low, medium and high concentrations in five replicates. The extraction recovery of the assay from human plasma was assessed by comparing the detector response for the extracted samples with those of the samples added at the same concentration after extracting the blank plasma. The recovery of the IS was evaluated at the working concentration (100 ng/mL) in the same manner.

Stability

The stability of paeoniflorin in the plasma sample was evaluated under different conditions including bench-top stability, processed sample stability and freeze–thaw stability. The stability tests were performed using the QC samples at two different concentrations: low (0.6 ng/mL) and high (16 ng/mL). The bench-top stability was conducted using the QC samples at room temperature for 8 h. The processed sample stability was determined by analyzing the extracted samples in the sample injector at 10 °C for 8 h. The freeze–thaw stability of the paeoniflorin was evaluated by the comparison of plasma samples after three freeze–thaw cycles at −80 °C and room temperature (20 °C) on the consecutive days. The compounds were considered to be stable if the variation of the assay values (n = 5) was <15 % of the initial time response.

Incurred sample reanalysis

An incurred sample reanalysis (ISR) was carried out by the computerized random selection of the human plasma samples (7 % of total samples analyzed). The selection criteria include the following: samples near the maximum concentration (C_{max}) and the elimination phase in the PK profile of the drug. The number of samples per human ranged from 1 to 2. A total of 120 samples were analyzed and nine samples were selected for the ISR. The results were compared to the data obtained earlier for the same sample using the same procedure. The percentage difference was calculated as the absolute difference divided by the mean of the initial value and the repeat value of each sample. Two-third (67 %) of the reassay sample results should be within 20 % (US Food and Drug Administration 2013).

Results and discussion

Content of paeoniflorin

The content of paeoniflorin ($C_{23}H_{28}O_{11}$, Molecular Weight: 480.47 g) in the Paeoniae Radix extract powder was calculated as 16.01 mg based on 0.5032 g of Paeoniae Radix extract powder.

Pharmacokinetics of paeoniflorin in humans

The plasma concentration–time relationship of paeoniflorin following a single usual 108.18 mg oral dose is illustrated in Fig. 2, and the relevant PK parameters including $AUC_{0-\infty}$, CL/F, V_d/F, C_{max}, T_{max} and $t_{1/2}$ are listed in Table 1. The best PK model of paeoniflorin in humans was
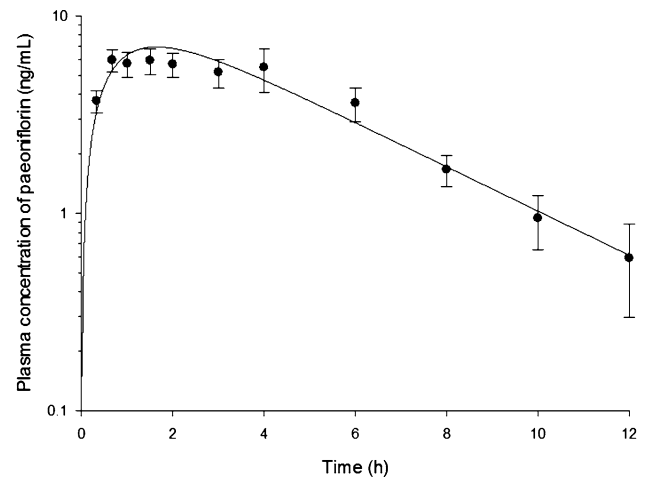

Fig. 2 Mean plasma concentration versus time profile of paeoniflorin following oral dosing of the dried powder of Paeoniae Radix extract (3.4 g containing 108.18 mg paeoniflorin) to healthy Korean subjects (n = 10)

Table 1 Pharmacokinetic parameters for paeoniflorin in humans after the usual oral dose administration of dried powder of Paeoniae radix extract (3.4 g containing 108.18 mg paeoniflorin) (mean ± SD, n = 10)

Parameters	Paeoniflorin	
	Estimate	CV (%)
$AUC_{0-\infty}$ (ng h/mL)	41.20 ± 2.01	4.88
CL/F (L/h)	2625.71 ± 128.16	4.88
V_d/F (L)	10,150.55 ± 1074.52	10.59
C_{max} (ng/mL)	6.97 ± 0.41	5.82
T_{max} (h)	1.64 ± 0.16	9.98
$t_{1/2}$ (h)	2.68 ± 0.22	8.26

the one compartment model with no lag time. The $AUC_{0-\infty}$, CL/F, and V_d/F were 41.20 ng h/mL, 2625.71 L/h, and 10,150.55 L, respectively. The C_{max} and T_{max} were 6.97 ng/mL and 1.64 h, respectively. Based on the previous studies in humans, the $t_{1/2}$ of paeoniflorin was found to be 2.4 ± 0.1 h in Chinese subjects (Li et al. 2014) and 1.74 h in Japanese subjects (Sadakane et al. 2015). The $t_{1/2}$ value (2.68 h) of this study was similar to others (Li et al. 2014; Sadakane et al. 2015).The T_{max} was reported in the literature 2–3 h (Sadakane et al. 2015), and the T_{max} value of our study was 1.64 h. There was no significant difference in terms of T_{max}. After the oral administration of 24.8 mg and 49.6 mg dose, the $AUC_{0-\infty}$ values were 34.7 and 39.5 ng h/mL, respectively (Sadakane et al. 2015). These results were similar to our study when we administered 108.18 mg dose of paeoniflorin in oral, and the proportionality between dose and $AUC_{0-\infty}$ was not shown. Based on the previous studies in Japanese and Chinese,

there were no significant difference in terms of the PK parameter value such as $t_{1/2}$, $AUC_{0-\infty}$, and T_{max} between Japanese and Chinese. As a result, no significant ethnic difference in Asian was observed on the PKs of paeoniflorin compared with other studies. This study could be important for the use of paeoniflorin in the Asian countries. This study is the first to report the PK parameters of paeoniflorin in Korean subjects after the usual oral dose administration of Paeoniae Radix extract powder and compare the Korean subjects with Japanese and Chinese subjects. The suitability of the developed analytical method was demonstrated in the PK study of paeoniflorin after a single usual oral dose administration of Paeoniae Radix extract powder (3.4 g containing 108.18 mg paeoniflorin) to 10 healthy Korean subjects.

UPLC-MS/MS conditions

During the UPLC-MS/MS assay development, the sensitivity, selectivity and specificity of the assay had been taken into the consideration for the determination of compounds in biological samples. In this study, mass spectrometric parameters such as capillary voltage, collision energy, desolvation temperature, ion source temperature, and flow rate of desolvation and cone gas were optimized for the determination of paeoniflorin. The maximum stable responses of the parent and product ions were obtained by directly infusing an individual standard solution of paeoniflorin with the mobile phase into the mass spectrometer. Under the ESI conditions selected, greater sensitivity was found for paeoniflorin and IS in the negative ESI mode using MRM. As a result, a good resolution and high intensity were obtained for quantitative measurement, and no cross-talk effect was observed produced by using different channels in the mass system. The full scan mass spectra of product ions for paeoniflorin and geniposide (IS) are shown in Fig. 3. The MRM transitions were at m/z 525.2 → 121.0 and 433.1 → 225.1 for paeoniflorin and geniposide (IS), respectively.

Chromatography

The composition effect of the mobile phase and the initial composition of the mobile phase were chose non the basis
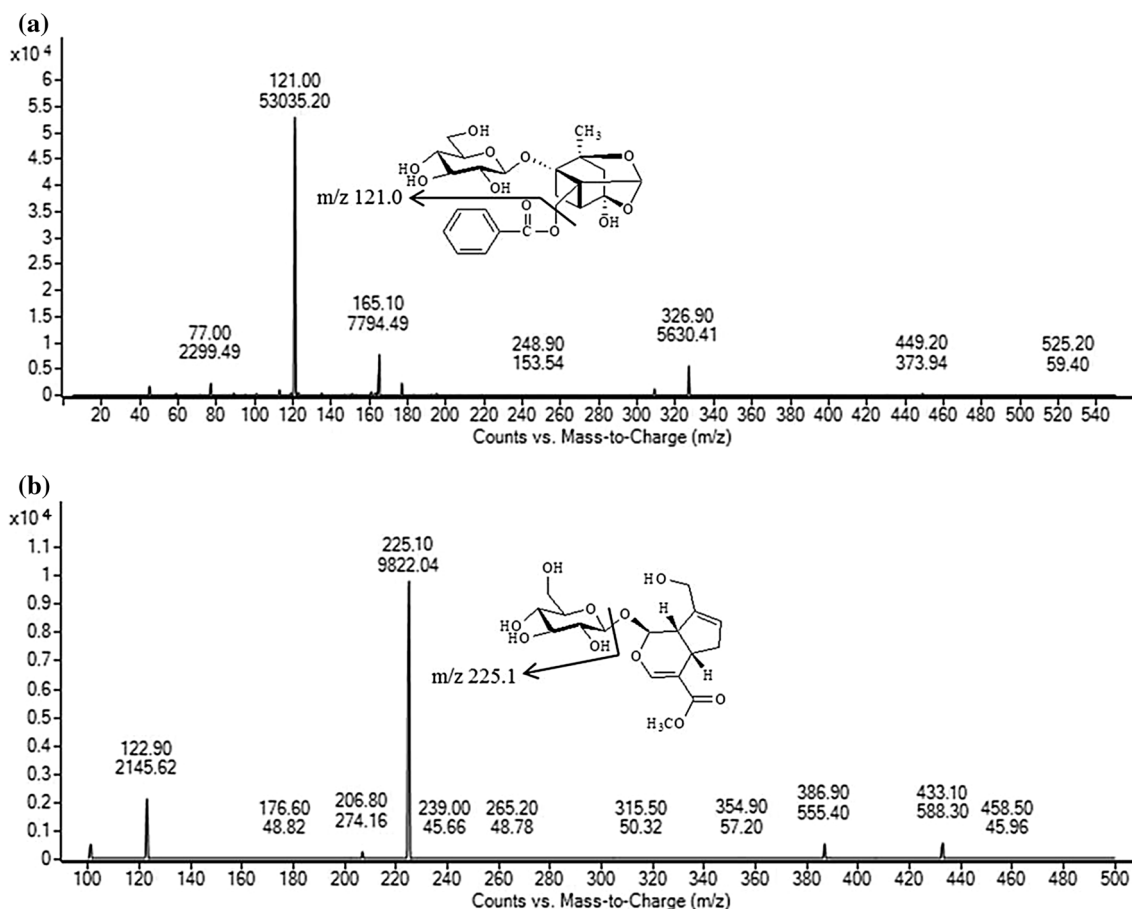

Fig. 3 Negative product ion mass spectra of **a** paeoniflorin and **b** geniposide (IS)

of the pKa values (4.4) of paeoniflorin (Zhou et al. 2010). Mobile phase was tried using buffers such as ammonium formate, ammonium acetate, as well as acid additives such as acetic acid and formic acid. The addition of formic acid to the mobile phase resulted in the most sensitive intensity of paeoniflorin. Acetonitrile showed the higher sensitive response and lower background noise than methanol and was then chosen as the organic solvent for the mobile phase. According to the result, acetonitrile and water containing 0.1 % formic acid were optimized as the mobile phase using gradient elution. The gradient elution satisfied regarding the peak shape, retention time and interference peaks separating paeoniflorin.

The most widely used sample preparation techniques in biological samples are plasma protein precipitation (PPT), liquid–liquid extraction (LLE), and solid-phase extraction (SPE). The LLE and PPT methods were tested. For LLE, ethyl acetate, ethyl ether, methylene chloride, and methyl-*tert*-butyl ether were attempted for extraction. Paeoniflorin was not extracted in any of the four selected organic solvents. Methanol showed the higher sensitive response than acetonitrile and was then chosen as the precipitation solvent for the PPT method. In the previous study, Li et al. (2014) reported an LLOQ of 0.6 ng/mL using PPTin human plasma. To evaluate the PKs of paeoniflorin after the usual oral dose administration, a sensitive analytical method of paeoniflorin in human plasma was required. In this study, the supernatant after the precipitation of protein by PPT was evaporated to dryness under a gentle nitrogen stream at 50 °C to improve the sensitivity of paeoniflorin. This study provided a higher sensitivity compared to the previous study with an LLOQ of 0.2 ng/mL. Moreover, our developed method was much simpler, less time-consuming and afforded higher recovery than LLE.

Method validation

Specificity

Figure 4 shows the representative chromatogram of the blank plasma (A), spiked plasma sample with paeoniflorin (0.2 ng/mL), IS (100 ng/mL) (B), and the plasma sample obtained from a subject 1.5 h after the oral administration of Paeoniae Radix extract powder (C). No significant chromatographic interference was observed with paeoniflorin and IS at their retention times in drug-free plasma. The retention times of paeoniflorin and IS were 2.56 and 1.61 min, respectively. This PPT procedure was considered to minimize the interfering peaks, in particular, for the analysis of paeoniflorin at a lower dose administration.

Linearity and LLOQ

The calibration curves for paeoniflorin in the human plasma exhibited a good linearity over the concentration range 0.2–20 ng/mL for paeoniflorin with a correlation coefficient (r^2) exceeding 0.991. The typical linear regression equations of the calibration curves are: $y = (0.41844 \pm 0.03604)\ x + (0.00438 \pm 0.00102)$ for paeoniflorin with y as the peak–area ratio of paeoniflorin to IS and x (ng/mL) as the plasma concentration of paeoniflorin. The developed UPLC-MS/MS analysis provided an LLOQ of 0.2 ng/mL, which was sufficient for PK study after the usual oral dose administration of Paeoniae Radix extract powder in humans.

Precision and accuracy

During the validation, good performance with a low deviation and consistent accuracy was investigated for four QC samples. Table 2 lists the intra- and inter-batch precision and accuracy for paeoniflorin of the LLOQ (0.2 ng/mL) and QC samples at 0.6, 3.2 and 16 ng/mL in the human plasma. All the values of CVs for the paeoniflorin ranged from 3.12 to 12.72 % within the acceptable criteria, and the accuracy was within the range from 92.31 to 101.87 %, suggesting that the method was accurate and reproducible for the determination of paeoniflorin in human plasma.

Recovery

The recovery of paeoniflorin from human plasma was 78.2 ± 4.4 %, whereas the recovery of IS was 84.0 ± 5.2 %. This simple PPT method was successfully applied to the determination of paeoniflorin in human plasma.

Stability

The results of the stability are listed in Table 3. The QC samples (low and high) of paeoniflorin were stable at room temperature (20 °C) for up to 8 h without any significant degradation; the REs were within 6.46 % for paeoniflorin. The processed sample stability was stable at autosampler (10 °C) up to 8 h, and the REs were within 12.31 %. Moreover, paeoniflorin was also considered to be stable after undergoing three freeze–thaw cycles in human plasma (the RE was <5.77 %). The paeoniflorin in human plasma was stable at 20 °C for up to 7 weeks (the RE was <4.63 %) and therefore was stable for the long term. The results showed that the analytes were stable under different storage conditions.
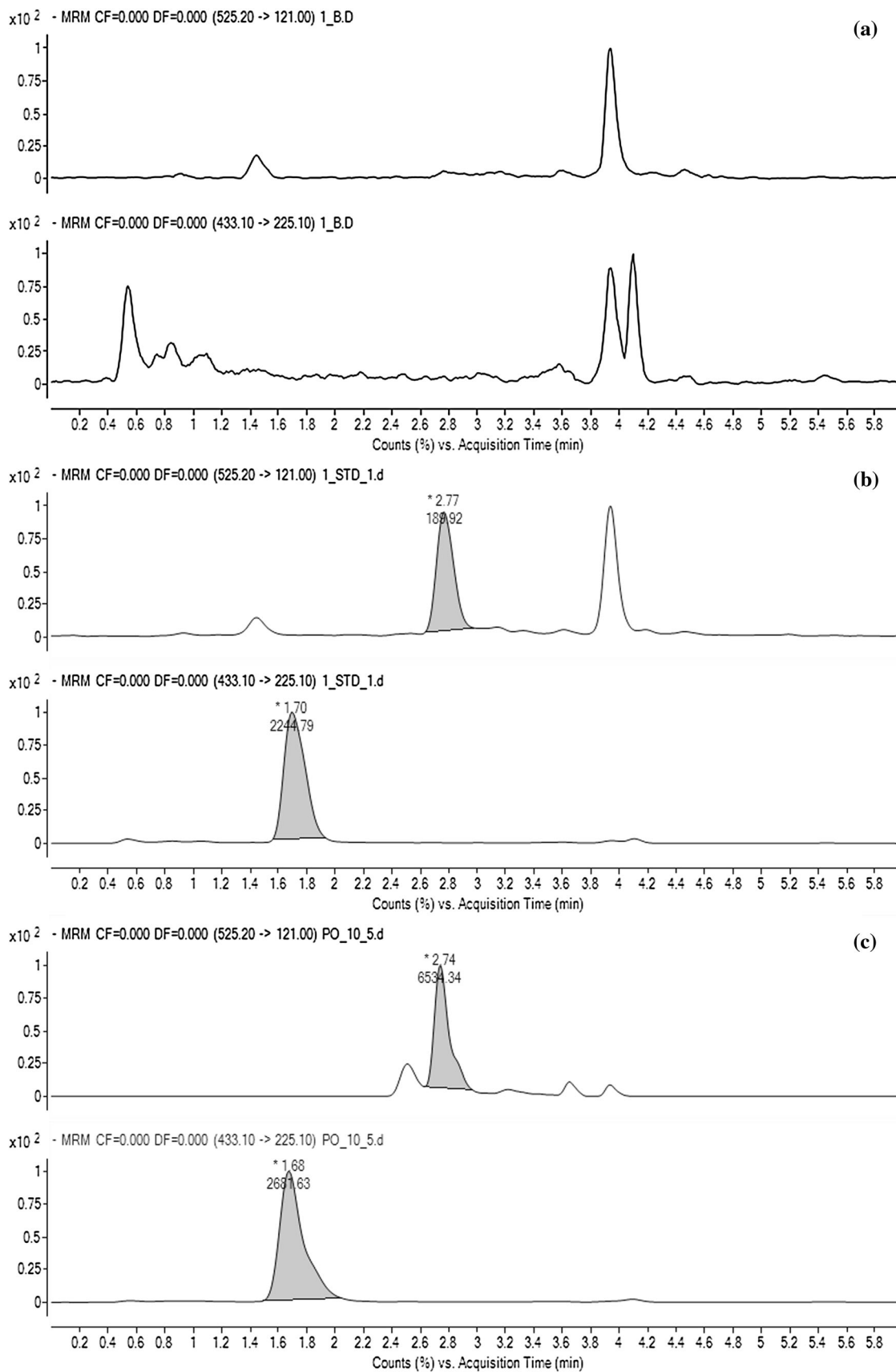
◄ **Fig. 4** Representative MRM chromatograms of **a** blank human plasma extract, **b** human plasma sample extract containing paeoniflorin at an LLOQ of 0.2 ng/mL and IS (100 ng/mL), **c** human plasma sample extract taken at 1.5 h after the usual oral dose administration of Paeoniae radix extract powder (3.4 g containing 108.18 mg paeoniflorin)

Table 2 Intra-day and inter-day precision and accuracy data for paeoniflorin in human plasma

Added concentration (ng/mL)	Measured (ng/mL, Mean ± SD)	Precision (CV, %)	Accuracy (%)
Intra-batch (n = 5)			
0.2 (LLOQ)	0.19 ± 0.02	12.72	92.31
0.6	0.61 ± 0.04	5.94	101.23
3.2	3.00 ± 0.10	3.23	93.58
16	15.32 ± 0.63	4.11	95.72
Inter-batch (n = 5)			
0.2 (LLOQ)	0.20 ± 0.02	11.19	101.30
0.6	0.59 ± 0.02	3.12	98.68
3.2	3.17 ± 0.14	4.42	99.03
16	16.30 ± 0.77	4.73	101.87

Table 3 Stability of paeoniflorin in human plasma under various storage conditions (n = 5)

Storage conditions	Concentrations (ng/mL)		RSD (%)	RE (%)
	Nominal	Measured		
Bench-top	0.6	0.55 ± 0.02	4.04	−6.46
	16	15.86 ± 0.70	4.42	−1.23
Processed sample	0.6	0.68 ± 0.01	1.75	12.31
	16	15.41 ± 0.51	3.28	−4.04
Freeze–thaw	0.6	0.61 ± 0.01	2.08	5.01
	16	16.99 ± 0.49	2.89	5.77
Long-term	0.6	0.61 ± 0.03	4.75	3.80
	16	16.80 ± 0.68	4.01	4.63

Incurred sample reanalysis

This assay was further evaluated using human samples in an ISR. The results of all the nine samples were within 15 % (US Food and Drug Administration 2013).

Conclusions

In conclusion, we first evaluated the PKs of paeoniflorin, after the usual oral dose of Paeoniae Radix extract powder (3.4 g containing 108.18 mg paeoniflorin) in Korean subjects. In addition, a selective and sensitive UPLC-MS/MS method was developed and validated for the quantification of paeoniflorin in the human plasma.

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