Journal of Chromatography B, 905 (2012) 54–60

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Simultaneous quantification of Polyphyllin D and Paris H, two potential antitumor active components in *Paris polyphylla* by liquid chromatography–tandem mass spectrometry and the application to pharmacokinetics in rats

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ARTICLE INFO

Article history:
Received 9 February 2012
Accepted 1 August 2012
Available online 8 August 2012

Keywords:
Paris polyphylla
Polyphyllin D
Paris H
Pharmacokinetics
LC–ESI-MS/MS

ABSTRACT

Polyphyllin D and Paris H are two potential antitumor active components in *Paris polyphylla*, one of the Traditional Chinese Medicines (TCMs). The present study details the development and validation of a rapid, sensitive and accurate LC–ESI-MS/MS method for the separation and simultaneous determination of Polyphyllin D and Paris H in rat plasma using Ginsenoside Rh_2 as the internal standard (IS). A simple protein precipitation method was used for the preparation of plasma sample. Chromatographic separation was successfully achieved on an Agilent Zorbax C_{18} column using a step gradient program with the mobile phase of 10 mmol/L aqueous ammonium acetate and acetonitrile. Both analytes were detected by negative mode electrospray ionization mass spectrometry. Selected reaction monitoring (SRM) was applied for all monitored analytes. This method demonstrated good linearity and did not show any endogenous interference. The lower limits of quantification (LLOQs) of Polyphyllin D and Paris H were both 1.0 ng/mL using 100 μL rat plasma. The average recoveries of Polyphyllin D and Paris H from rat plasma were both above 80%. The inter-day precisions (%RSD) of both analytes determined in five days were all below 15%. The developed and validated method has been successfully applied in the simultaneous quantification and pharmacokinetic studies of Polyphyllin D and Paris H in rats.

1. Introduction

Paris polyphylla Smith var. *yunnanensis* (Fr.) Hand.-Mazz. (PPY), mostly distributes in southwest China, is a well-known Traditional Chinese Medicine (TCM) and has been documented in Chinese Pharmacopoeia (ChP). *P. polyphylla* has been used for a long time in China as an important herbal drug for the treatment of inflammation, fractures, parotitis, hemostasis, snake bite and abscess in folk medicines. In recent years, it is playing a more and more important role in the medicine development for anti-cancer, immunity adjustment and anti-inflammation [1]. Extensive phytochemistry and pharmacological studies have further identified Polyphyllin D and Paris H, both of which belong to steroidal saponins and are considered to be the potential antitumor active components in *P. polyphylla* [2–6].

Both the *in vitro* and *in vivo* research results have shown that Polyphyllin D is a potent apoptosis inducer through mitochondrial dysfunction in drug-resistant HepG2 cells and also exerts potent anti-antigenic effects [7–9]. In addition, *in vivo* studies indicated that Polyphyllin D is effective in reducing the tumor size of human breast tumor xenografts in nude mice without apparent toxicity to the host and inhibited P-glycoprotein-mediated anti-tumor drug efflux in multidrug resistant human leukemia cells [10,11]. In our previous work, preliminary drug screening revealed that Polyphyllin D and Paris H showed potential anticancer activities against lung adenocarcinoma *in vivo*, including a powerful antiproliferative effect. It was demonstrated that the Polyphyllin D and Paris H could induce apoptosis and inhibit pulmonary metastasis by reducing the expression of MMP-2 and MMP-9 [3,12], and both

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1570-0232/$ – see front matter.
http://dx.doi.org/10.1016/j.jchromb.2012.08.002

of which could also inhibit the metastasis of B16 melanoma cells [13]. Moreover, Paris H also displayed some strong cytotoxicity to mouse lung cancer LA795 cells and A549 cells [14–16].

Due to the above potential biological effects, it is necessary to carry out the bioanalysis and pharmacokinetic studies on Polyphyllin D and Paris H. The *in vitro* quantitative methods of Polyphyllin D and Paris H analysis have been carried out in the last several years mainly by liquid chromatography with both reversed phase columns coupled to diode array detector (DAD) and evaporative light scattering detector (ELSD) [17–20]. None of these methods meets the requirements for the analysis of biological fluids with respect to an efficient clean-up procedure, shorter running time and higher sensitivity. Furthermore, there is no published analytical method for simultaneous quantification of Polyphyllin D and Paris H in biological fluids. Therefore, methodological development and validation for the simultaneous quantitative analysis of Polyphyllin D and Paris H in biological fluids are required to support preclinical and further clinical trials aiming at determining oral bioavailability, defining pharmacokinetic and safety profiles and establishing optimal doses for maximum efficacy.

The aim of this study was to develop an accurate, sensitive and rapid LC–ESI-MS/MS method for the simultaneous quantification of Polyphyllin D and Paris H in rat plasma. Furthermore, the developed method was fully validated and applied to the pharmacokinetic study of Polyphyllin D and Paris H in rats thereafter.

2. Experimental

2.1. Materials and reagents

Polyphyllin D (batch No. 111591-2004402) and Ginsenoside Rh_2 (internal standard, IS, batch No. 110703-200424) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Paris H was purchased from Kobeyuan Biomedical Technology Company (Beijing, China). The purities of these standards were determined to be more than 99% by normalization of the peak areas detected by HPLC–UV. *P. polyphylla* Smith var. *yunnanensis* was collected in September 2010 from Lijiang (Yunnan, China) and identified by Professor Wenyuan Gao. The extract of *P. polyphylla* (containing 10.1% (w/w) Polyphyllin D and 8.60% (w/w) Paris H) was prepared in our laboratory according to the published procedures [3]. Acetonitrile (HPLC grade) was obtained from Merck (Darmstadt, Germany). Ammonium acetate was purchased from Tedia (Fairfield, USA). Ultrapure water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA). All other chemicals were of analytical grade.

2.2. Equipments and chromatographic–mass spectrometric conditions

The HPLC system consisted of an LC-20AD pump, a DGU-20 A_3 degasser, an SIL-20AC autosampler and a CTO-20A column oven (Shimadzu, Japan). The HPLC separation was performed on an Agilent Zorbax C_{18} column (150 mm × 2.1 mm i.d., 5 μm particle size) with a gradient elution by a mobile phase consisting of acetonitrile (A) and water (B) containing 10 mmol/L ammonium acetate with following gradient: 0 min (A 30%–B 70%) to 0.7 min (A 30%–B 70%) to 4.30 min (A 95%–B 5%) to 4.60 min (A 5%–B 95%) to 6 min (A 30%–B 70%), with the flow rate of 0.4 mL/min. The injection volume was 10 μL. The HPLC system was coupled with an API 4000 Qtrap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) *via* a Turbo IonSpray ionization interface. Following optimization of the setting parameters, the instrument was operated in the negative mode with an ion spray voltage of −4.5 kV, curtain gas pressure of 10 psi, nebulizer gas pressure of 30 psi, heater gas pressure of 15 psi and the source temperature set at 500 °C. The curtain, nebulizer, heater and collision gases were all nitrogen. The selected reaction monitoring (SRM) conditions were m/z 853.6 → 721.9, m/z 869.0 → 737.2 and m/z 621.8 → 161.0 for Polyphyllin D, Paris H and IS, respectively. The detection parameters were optimized as follows: collision energy, −40 eV for Polyphyllin D, −42 eV for Paris H and −31 eV for the IS; declustering potential (DP), −140 eV for Polyphyllin D, −120 eV for Paris H and −138.4 eV for the IS. The data were collected and analyzed by the Analyst Data Acquisition and Processing software (Version 1.5.1, Applied Biosystems/MDS Sciex).

2.3. Animals

Five male Sprague-Dawley rats (weighing 220 ± 30 g, 8 weeks) were purchased from Tianjin Medical Institute (Tianjin, China). The animals were housed in an air-conditioned room at a temperature of 23 ± 2 °C, with a relative humidity of 55 ± 10%, an illumination intensity of 150–300 lx, a frequency of air ventilation of 15–20 times/h and a 12 h illumination. Food and water were supplied *ad libitum*. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethic Committee of Tianjin University.

2.4. Preparation of stock and working solutions

The reference standards of Polyphyllin D and Paris H were accurately weighed and dissolved in methanol, and then diluted to appropriate concentrations using methanol for establishment of calibration curves in rat plasma. The concentrations of stock solutions of two reference standards were both 1.0 μg/mL. Working solutions of these two active compounds were prepared at concentrations of 2.0, 4.0, 10, 20, 50, 100, 200 and 500 ng/mL for Polyphyllin D and Paris H, respectively. The IS (Ginsenoside Rh_2) working solutions (800 ng/mL) were prepared by diluting with methanol. All the stock and working standard solutions were stored under refrigeration at 4 °C prior to use.

2.5. Preparation of calibration standards and quality control (QC) samples

Calibration curves and low, medium, high quality control (QC) samples of Polyphyllin D and Paris H were prepared by spiking 50 μL of working standard solutions and 50 μL of IS to 100 μL of blank rat plasma (drug-free). Calibration standards were prepared at plasma concentrations of 1.0, 2.0, 5.0, 10, 25, 50, 100 and 250 ng/mL for both analytes, while QC samples were prepared at 5.0, 25, 200 ng/mL for both analytes to evaluate the precision, accuracy, stability and recovery of analytical method, and then treated as described above.

2.6. Sample treatment

After thawing of the plasma samples at room temperature for about 30 min and vortexing for 30 s, aliquots of 100 μL plasma was mixed with 200 μL of IS solutions and 50 μL of methanol (or standard or QC solution) in 1.1 mL tubes. After vortexing for 1 min and centrifuging at 12,000 × *g* for 10 min, the supernatant was transferred to another tube and evaporated at 40 °C under a gentle stream of nitrogen until dry. The residues were dissolved in 100 μL of the mixture of methanol and water (75:25, v/v), and then transferred to HPLC vials. A volume of 10 μL of this solution was then injected into the column.

2.7. Method validation

The method was validated for the selectivity, matrix effect, linearity, lower limits of quantification (LLOQs), accuracy, precision, recovery and stability. To evaluate the selectivity, five individual samples of blank rat plasma were analyzed by comparing with the plasma-spiked analytes for endogenous interferences. Each blank sample was tested by developed LC–ESI-MS/MS method for potential interferences. To evaluate the matrix effects, chromatographic peak of Polyphyllin D and Paris H and IS from the spike-after prepared samples were compared with those of the neat standards in mobile phase at the QC concentrations.

The linearity of the method was determined by analyzing a series of standard plasma samples at concentrations of 1.0, 2.0, 5.0, 10, 25, 50, 100 and 250 ng/mL for both analytes by least squares linear regression of the peak area ratios of each analyte to IS obtained against the corresponding concentrations (C) with a weighting factor of $1/C^2$. The LLOQ was defined as the lowest concentration on the calibration curve with acceptable precision and accuracy. The criteria for the calibration included a correlation coefficient (r) of 0.995 or better. Concentrations in the QCs and unknown plasma samples were quantified by using the internal standard calibration method.

The precision and accuracy of method were assessed by performing replicate analyses of QC samples spiked with low, medium and high concentrations against calibration standards. Five replicates of QC samples at each concentration level were evaluated on the same day for intra-day precision, while repeated analysis at each concentration of QC samples five times per day over five consecutive days for inter-day precision and accuracy. Standard deviation (SD) and relative standard deviation (%RSD) were calculated from the QC values and used to estimate the inter- and intra-day precision.

Recoveries of Polyphyllin D and Paris H from rat plasma were determined by comparing the responses of Polyphyllin D and Paris H and IS in plasma carried through the complete preparation procedure to those spiked into the prepared blank plasma of the same concentration as those of QC samples, respectively.

The stability of analytes in rat plasma was assessed by analyzing QC samples at two concentrations exposed to different time and temperature conditions. The long-term stability was assessed after the QC samples had been stored at −80 °C for 1 month. The freeze–thaw stability was determined after three freeze–thaw cycles (−20 °C to 20 °C) on 3 consecutive days. The amount of the compounds in plasma samples was determined using a newly prepared calibration curve. Stability of analytes was expressed as a percentage of nominal concentration. Deviation of the stability results should be within ±15% of the nominal values.

2.8. Pharmacokinetic study in rats

The rats were fasted for 16 h before experiments with the exception of free access to water. The dosing solution was prepared by dissolving appropriate amount of the extract of *P. polyphylla* in normal saline (0.5% NaCl solution). After a 2-day recovery from femoral vein cannulated surgery, the animals were administered the extract of *P. polyphylla* by oral gavages. The actual doses of Polyphyllin D and Paris H were 35 mg/kg and 30 mg/kg converted according to their contents in the extracts, respectively. After oral administration, aliquots of 0.25 mL blood samples were collected in heparinized polyethylene tubes at different time intervals postdosing (1.5, 2.5, 3.5, 4.5, 8, 10, 12, 24, 36 and 48 h). Heparinized blood was centrifuged at 12,000 × g at room temperature for 5 min to obtain plasma, which was stored at −80 °C until analysis.

Data fitting and pharmacokinetic parameter calculations were carried out using the DAS 2.1 pharmacokinetic program in which the non-compartmental model was chosen. C_{max} and T_{max} values were obtained directly from the observed concentration *versus* time data. All results were expressed as arithmetic mean ± standard deviation (SD).

3. Results and discussion

3.1. Optimization for LC–MS/MS parameters

To optimize the MS conditions, both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) modes were investigated. When APCI mode was used, no specific and abundant ions for these two analytes of interest were observed in either positive or negative mode probably because these two compounds possess high polarity and/or weak lipophilicity. In the negative mode of ESI, $[M-H]^-$ ions of Polyphyllin D and Paris H were formed at high abundance and the full-scan product ion spectra are shown in Fig. 1. The precursor–product ion reactions selected were m/z 853.6 → 721.9, m/z 869.0 → 737.2 and m/z 621.8 → 161.0 for Polyphyllin D, Paris H and IS, respectively. Other conditions such as ion spray voltage, curtain gas pressure, nebulizer gas pressure, heater gas pressure, the source temperature and collision energy were further optimized.

During the optimization of chromatographic conditions, Polyphyllin D, Paris H and IS were extensively retained on several kinds of columns. To achieve symmetric peak shapes and short chromatographic running times, the mobile phase consisting of acetonitrile and aqueous ammonium acetate was used on a Zorbax C_{18} column.

An internal standard is usually required in LC–MS/MS analysis. In this study, Ginsenoside Rh_2, a readily available compound with similar chemical structure with those of two analytes of interest, was selected as the IS; its chromatographic retention behavior and extraction efficiency were similar to those of the two analytes. In addition, there were no interferences from the two analytes and endogenous substances.

3.2. Selectivity

The selectivity of the method was examined by comparing the blank plasma and spiked plasma (n = 5). Under the above conditions the retention time was about 3.32 min, 2.85 min and 3.84 min for Polyphyllin D, Paris H and IS, respectively. As shown in Fig. 2, no obvious interferences from endogenous substances were observed in the representative chromatograms of blank plasma samples at the retention times of the analytes and the IS.

3.3. Matrix effect and recovery

The mean absolute matrix effect values obtained were 101% and 97.6% for Polyphyllin D and Paris H, respectively (Table 1). The results indicated that ion suppression or enhancement from plasma matrix was negligible for this current analytical method.

The recoveries were determined in five replicates of rat plasma spiked with low, medium and high concentrations of Polyphyllin D and Paris H, respectively. The results are summarized in Table 1. The mean recoveries of Polyphyllin D and Paris H were all above 80%. The data indicated that the recoveries of the analytes and IS from rat plasma were concentration-independent in the concentration range evaluated and the recoveries were acceptable for the pharmacokinetic analysis.

3.4. Linearity and lower limits of quantification (LLOQs)

The calibration curves calculated in the range of 1.0–250 ng/mL were linear to analyze Polyphyllin D and Paris H from rat plasma.

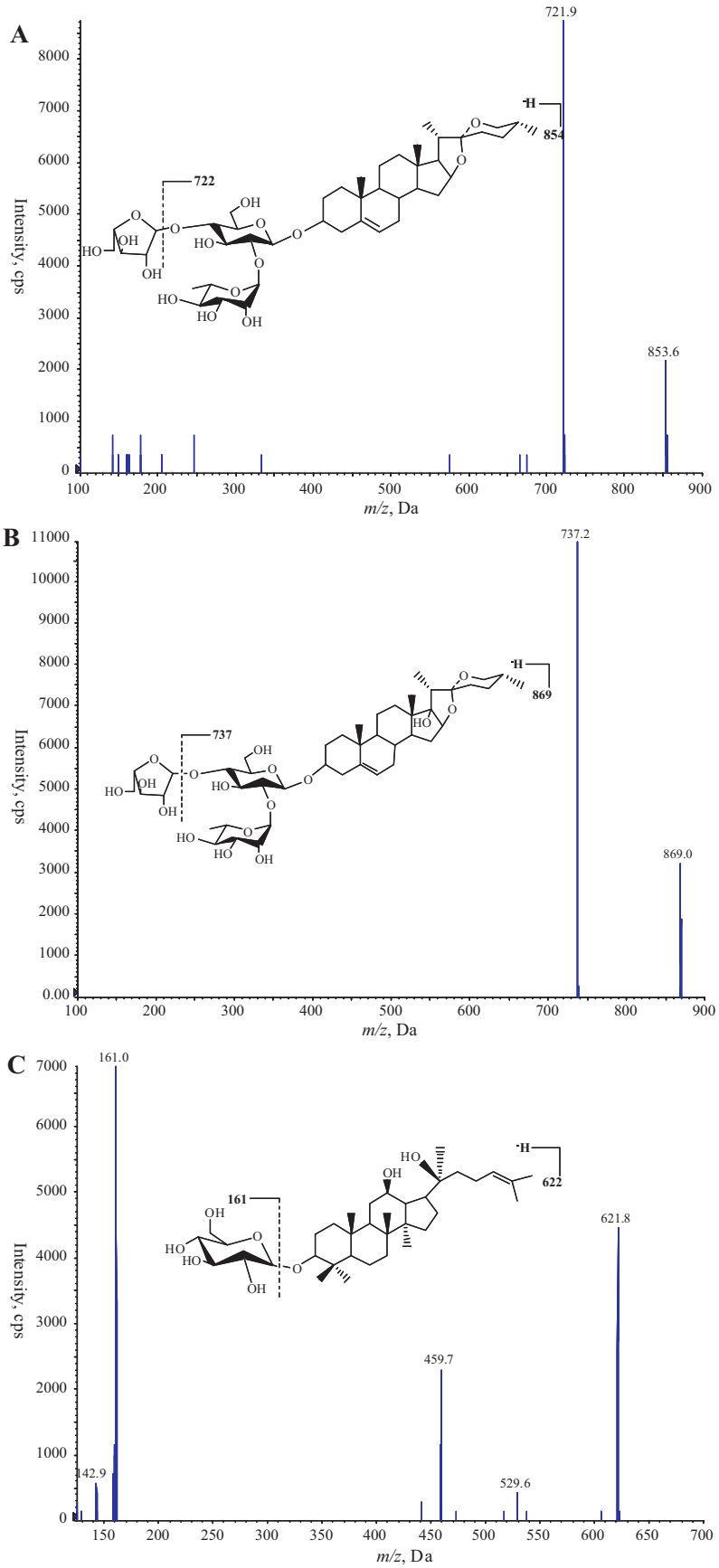

Fig. 1. Full-scan product ion spectra of $[M-H]^-$ ions and fragmentation schemes for (A) Polyphyllin D, (B) Paris H and (C) Ginsenoside Rh_2 (internal standard).

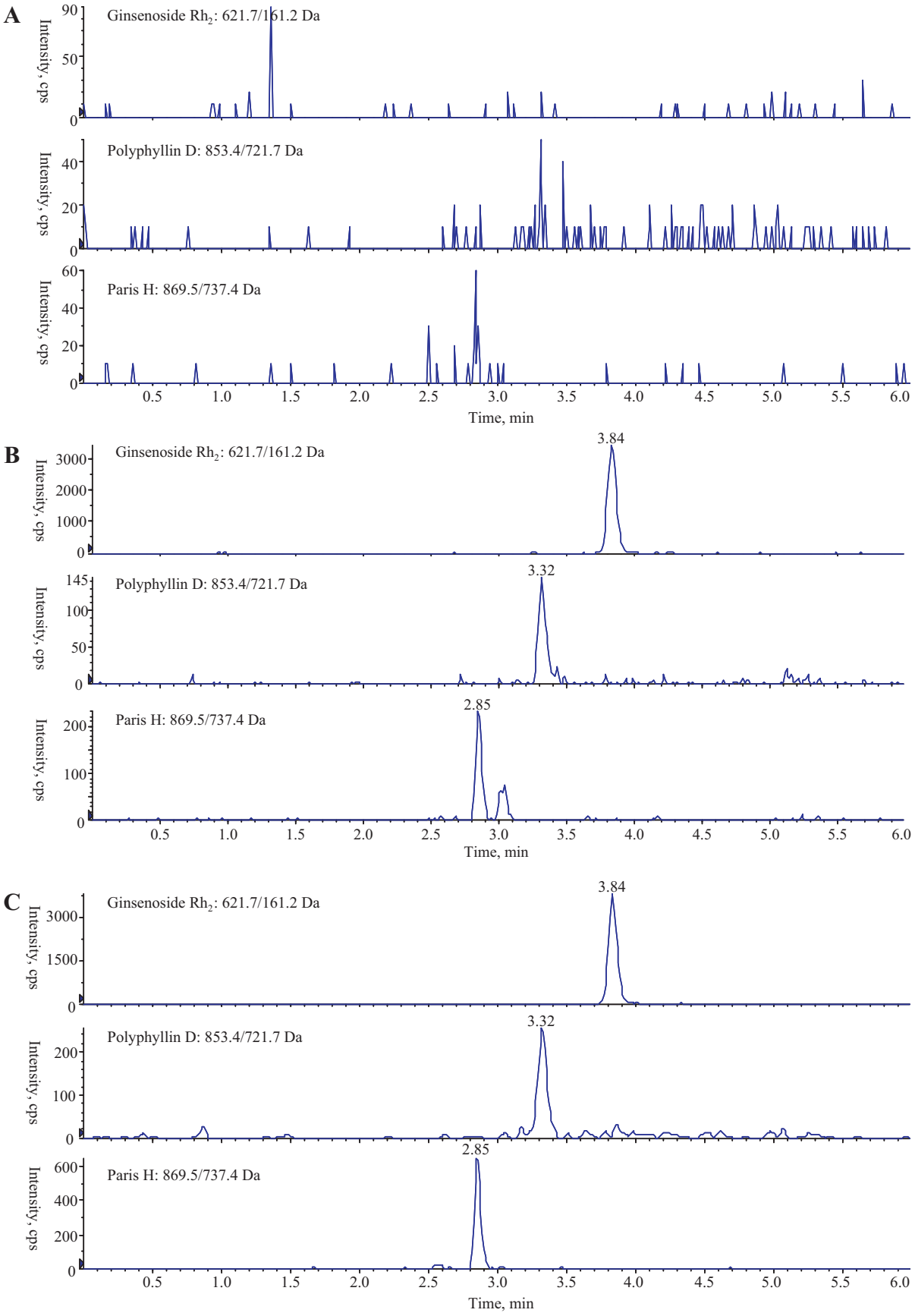

Fig. 2. Typical chromatograms of (A) blank rat plasma; (B) blank rat plasma spiked with Polyphyllin D (1.0 ng/mL), Paris H (1.0 ng/mL) and IS and (C) an unknown rat plasma sample collected at 8 h after oral administration of the extract of *Paris polyphylla*.

Table 1
Matrix effects and recoveries of Polyphyllin D and Paris H in rat plasma ($n = 5$).

Analytes	Spiked concentration (ng/mL)	Matrix effect (%)	Mean ± SD (%)	Recovery (%)	RSD (%)
Polyphyllin D	5.00	98.2	101 ± 5.11	102	5.73
	25.0	107		104	9.65
	200	98.1		98.6	4.41
Paris H	5.00	106	97.6 ± 7.28	104	6.93
	25.0	94.2		103	7.93
	200	92.7		96.4	6.34

Table 2
Intra-and inter-day precisions and accuracies of Polyphyllin D and Paris H in rat plasma ($n = 5$).

Analyte	Added (ng/mL)	Intra-day precision		Inter-day precision		Accuracy RE (%)
		Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	
Polyphyllin D	5.00	5.10	3.73	5.20	9.30	4.00
	25.0	23.1	5.21	24.9	7.20	−0.40
	200	216	12.2	194	10.2	−3.00
Paris H	5.00	4.80	4.30	4.80	6.51	−4.00
	25.0	25.9	3.40	25.2	6.17	0.80
	200	190	4.09	193	4.83	−3.50

Table 3
Stability results of Polyphyllin D and Paris H in rat plasma and stock solutions ($n = 5$).

Analyte	Conc. (ng/mL)	Stability					
		Three freeze–thaw cycles		Storage at −80 °C for 1 month		Room temperature for 24 h	
		Conc. (mean ± SD, ng/mL)	RE (%)	Conc. (mean ± SD, ng/mL)	RE (%)	Conc. (mean ± SD, ng/mL)	RE (%)
Polyphyllin D	5.00	5.50 ± 0.20	10.0	5.50 ± 0.20	10.0	5.50 ± 0.15	10.0
	25.0	27.1 ± 0.46	8.40	25.8 ± 0.61	3.20	26.9 ± 0.50	7.60
	200	203 ± 2.81	1.50	197 ± 1.63	−1.50	193 ± 4.11	−3.50
Paris H	5.00	5.20 ± 0.27	4.00	5.20 ± 0.30	4.00	5.20 ± 0.22	4.00
	25.0	25.3 ± 0.89	1.20	25.7 ± 0.80	2.80	25.1 ± 0.38	0.40
	200	206 ± 2.40	3.00	213 ± 2.60	6.50	199 ± 5.20	−0.50

The slopes, intercepts, and correlation coefficients of the regression equations were determined by least squares linear regression using an eight-factor of $1/x^2$. Typical equations for the standard curves were $y = 0.00762x - 0.00391$ ($r = 0.9983$) for Polyphyllin D and $y = 0.00856x - 0.000607$ ($r = 0.9952$) for Paris H. Deviations were within ±15% for all regression equations. The lower limits of quantification (LLOQs) were both 1.0 ng/mL for Polyphyllin D and Paris H.

3.5. Precision and accuracy

Table 2 summarized the intra- and inter-day precisions and accuracies of Polyphyllin D and Paris H at different concentration levels. As shown in Table 2, the intra- and inter-day accuracies of Polyphyllin D and Paris H (%RE) were within the range of ±15%. The intra- and inter-day precisions (%RSD) were all less than 15%. The results demonstrated that the values were all within the acceptable range and the method was proved to be accurate and precise.

3.6. Stability

The stability results are presented in Table 3. The results indicated that Polyphyllin D and Paris H at the three concentrations studied had acceptable stabilities after three cycles of freeze–thaw, at room temperature for 24 h and at −80 °C for 1 month with the %RE values being 1.20–10.0%, −3.50% to 10.0% and −1.50% to 10.0%, respectively.

3.7. Pharmacokinetics in rats

The validated method showed satisfactory results for the separation and simultaneous determination of Polyphyllin D and Paris H in rat plasma and was successfully utilized for the pharmacokinetic study of these two components after a single oral administration to rats. The plasma concentration–time profiles for Polyphyllin D and Paris H to rats are shown in Fig. 3 and the main pharmacokinetic parameters are presented in Table 4. It can be seen from Fig. 3 that Polyphyllin D and Paris H were eliminated slowly in rats and the drug plasma levels maintained low. The plasma concentration maximum of Polyphyllin D was 11.5 ± 0.50 ng/mL occurring at 8 h post dose, while the plasma concentration maximum of Paris H was

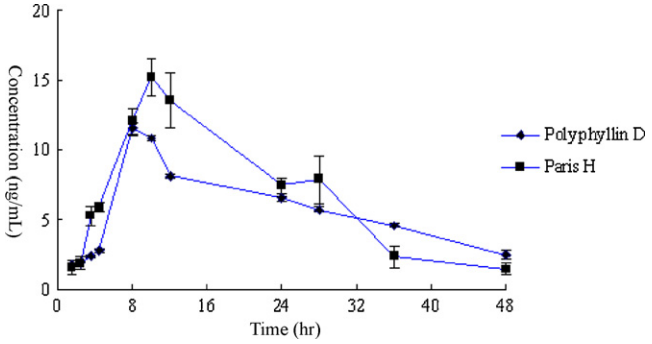

Fig. 3. Mean plasma concentration–time profiles of Polyphyllin D and Paris H determined by LC–ESI-MS/MS method after oral administration of the extract of *Paris polyphylla* to rats. Each point represents mean ± SD ($n = 5$).

Table 4
Main pharmacokinetic parameters of Polyphyllin D and Paris H in rats after oral administration of the extract of *Paris polyphylla* ($n = 5$, mean ± SD).

Parameter	Unit	Polyphyllin D	Paris H
AUC_{0-t}	ng h/mL	272 ± 3.10	316 ± 33.0
$AUC_{0-\infty}$	ng h/mL	369 ± 46.8	339 ± 42.9
$MRT_{0-\infty}$	h	39.7 ± 7.80	20.9 ± 1.40
$t_{1/2}$	h	24.3 ± 6.10	10.4 ± 2.10
T_{max}	h	8.00 ± 0.00	10.0 ± 0.00
C_{max}	ng/mL	11.5 ± 0.50	15.2 ± 1.40
CL	L/h/kg	52.3 ± 6.00	119 ± 15.1

$t_{1/2}$, half-life; AUC, area under the curve; MRT, mean residence time; T_{max}, time of maximum concentration; C_{max}, maximum concentration; CL, clearance.

15.2 ± 1.40 ng/mL occurring at 10 h post dose. Further researches on absorption, distribution, excretion and metabolism of Polyphyllin D and Paris H will be studied in our future studies.

4. Conclusion

An accurate, sensitive and rapid LC–ESI-MS/MS method with simple protein precipitation was developed and validated for the simultaneous determination of Polyphyllin D and Paris H in rat plasma. The established analytical method has been successfully applied to the pharmacokinetic studies of Polyphyllin D and Paris H in rats after oral administration with excellent sensitivity, good linearity of responses, high precision and accuracy.

Acknowledgement

This work was supported by a grant 2009ZX09103-362 Drug Innovation Project from Science and Technology in China.

References

[1] L.L. Yan, Y.J. Zhang, W.Y. Gao, S.L. Man, China J. Chin. Mater. Med. 33 (2008) 2057.
[2] Z.X. Cheng, B.R. Liu, X.P. Qian, Y.T. Ding, W.J. Hu, J. Sun, L.X. Yu, J. Ethnopharmacol. 120 (2008) 129.
[3] S.L. Man, W.Y. Gao, Y.J. Zhang, L.L. Yan, C.Y. Ma, C.X. Liu, L.Q. Huang, Steroids 74 (2009) 1051.
[4] Y. Wang, W.Y. Gao, X.Q. Liu, Y.T. Zuo, H.X. Chen, H.Q. Duan, Asian J. Tradit. Med. 1 (2006) 7.
[5] Y. Zhao, L.P. Kang, Y.X. Liu, Y.G. Liang, D.W. Tan, Z.Y. Yu, Y.W. Cong, B.P. Ma, Planta Med. 75 (2009) 356.
[6] L.L. Yan, Y.J. Zhang, W.Y. Gao, S.L. Man, Y. Wang, Exp. Oncol. 31 (2009) 27.
[7] R.C.Y. Ong, J. Lei, R.K.Y. Lee, J.Y. Cheung, K.P. Fung, C. Lin, H.P. Ho, B. Yu, M. Li, S.K. Kong, Cancer Lett. 261 (2008) 158.
[8] F.M. Siu, D.L. Ma, Y.W. Cheung, C.N. Lok, K. Yan, Z. Yang, M. Yang, S. Xu, B.C.B. Ko, Q.Y. He, C.M. Che, Proteomics 8 (2008) 3105.
[9] J.Y. Chan, J.C. Koon, X. Liu, M. Detmar, B. Yu, S.K. Kong, K.P. Fung, J. Ethnopharmacol. 137 (2011) 64.
[10] M.S. Lee, J.C. Yuet-Wa, S.K. Kong, B. Yu, V.O. Eng-Choon, H.W. Nai-Ching, T.M. Chung-Wai, K.P. Fung, Cancer Biol. Ther. 4 (2005) 1248.
[11] O. Noguer, J. Villena, J. Lorita, S. Vilaro, M. Reina, Exp. Cell Res. 315 (2009) 795.
[12] S.L. Man, W.Y. Gao, Y.J. Zhang, C.Y. Ma, L. Yang, Y.W. Li, Arch. Pharm. Res. 34 (2011) 43.
[13] S.L. Man, W.Y. Gao, Y.J. Zhang, Z. Liu, C.X. Liu, J. Ethnopharmacol. 3 (2011) 1221.
[14] W.S. Zhao, W.Y. Gao, J.C. Wei, Y. Wang, L.Q. Huang, P.G. Xiao, Lat. Am. J. Pharm. 30 (2011) 1702.
[15] Y. Wang, Y.J. Zhang, W.Y. Gao, L.L. Yan, China J. Chin. Mater. Med. 32 (2007) 1425.
[16] X.X. Huang, W.Y. Gao, S.L. Man, Y. Gao, L.Q. Huang, C.X. Liu, Lat. Am. J. Pharm. 29 (2010) 401.
[17] S.L. Man, W.Y. Gao, Y.J. Zhang, X.H. Jin, C.Y. Ma, X.X. Huang, Q.Y. Li, Anal. Bioanal. Chem. 395 (2009) 495.
[18] J.T. Lin, Y.Z. Chang, M.P. Lu, D.J. Yang, J. Agric. Food Chem. 59 (2011) 1587.
[19] K. Li, Y.W. Wang, J.K. Gu, X.Y. Chen, D.F. Zhong, J. Chromatogr. B 817 (2005) 271.
[20] S.L. Man, W.Y. Gao, Y.J. Zhang, J.Y. Wang, W.S. Zhao, L.Q. Huang, C.X. Liu, J. Chromatogr. B 878 (2010) 2943.