Short communication

Determining the pharmacokinetics of psilocin in rat plasma using ultra-performance liquid chromatography coupled with a photodiode array detector after orally administering an extract of *Gymnopilus spectabilis*

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ABSTRACT

This study established ultra-performance liquid chromatography coupled with a photodiode array detector for determining psilocin and its pharmacokinetics in rat plasma after orally administering an extract of *Gymnopilus spectabilis*. The extract was separated on an ODS C18 column (2.3 μm, 100 mm × 2.1 mm I.D.) by gradient elution with (A) water containing 50 mM $AcONH_4$ and (B) acetonitrile. The wavelength was set at 265 nm and the injection volume was 10 μL. Under these conditions, the calibration curve was linear over the concentration range 0.2–20 μg/mL with a correlation coefficient of r^2 = 0.9992. The inter- and intraday precision levels were less than 7% and the accuracies (%) were within the range 92.0–102.5%. The method was sufficiently valid to be applied to a pharmacokinetics study of psilocin in rat plasma. The pharmacokinetic parameters of psilocin in rat plasma after the oral administration of a *G. spectabilis* extract were as follows: C_{max}, 0.43 ± 0.12 μg/mL; T_{max}, 90 ± 2.1 min; $AUC_{0 \rightarrow t}$, 1238.3 ± 96.4 (μg/mL) min; and $T_{1/2}$, 117.3 ± 40.3 min.

1. Introduction

Psilocin (4-hydroxy-*N*,*N*-dimethyltryptamine) and its phosphoryloxy derivative psilocybin (4-phosphoryloxy-*N*,*N*-dimethyltryptamine) are indoles that occur naturally in hallucinogenic mushrooms belonging to the genus *Psilocybe* [1–4]. The psilocin and psilocybin in fungus material have been quantified using high-performance liquid chromatography (HPLC) with ultraviolet (UV), electrochemical (EC), and native fluorescence (FL) detection [5–9]. The metabolism of psilocybin was first investigated in rats using paper chromatography [10,11] and HPLC [12] and later in humans using gas chromatography (GC) and HPLC [13]. Ultra-performance liquid chromatography (UPLC) coupled with a photodiode array (PDA) detector is a new technique. It uses a sub-2-μm particle short column to enhance the retention time reproducibility, along with high chromatographic resolution, sensitivity, and high operating speed of UPLC [14–17].

Gymnopilus spectabilis (Fr.) Singer, a bitter, poisonous mushroom belonging to the family Strophariaceae, grows in dense clusters on stumps and logs of hardwoods and conifers. It contains the hallucinogenic alkaloid psilocin, which is toxic if ingested by humans [18–21]. The mushroom, called "magic mushroom," is sold legally. The small amounts of psilocin (a major active constituent) and psilocybin in the mushrooms induce ecstasy, but an overdose or ingestion with other drugs can be fatal. Some research has indicated that psilocybin acts as a prodrug and that its hydroxy metabolite psilocin is the true pharmacologically active agent [21,22]. Few studies, however, have investigated the pharmacokinetics of psilocin in rat plasma after the oral administration of *G. spectabilis*.

This study developed UPLC coupled with a photodiode array (PDA) detector to study the pharmacokinetic behavior of psilocin after the oral administration of *G. spectabilis* to rats. A study of the pharmacokinetics parameters of psilocin should provide useful information for clinical reference.

2. Materials and methods

2.1. Chemicals and materials

The HPLC-grade methanol and acetonitrile were purchased from Sigma (St. Louis, MO, USA). Ultrapure water was used for all analyses. Psilocin and baicalein standards (Fig. 1) were also purchased
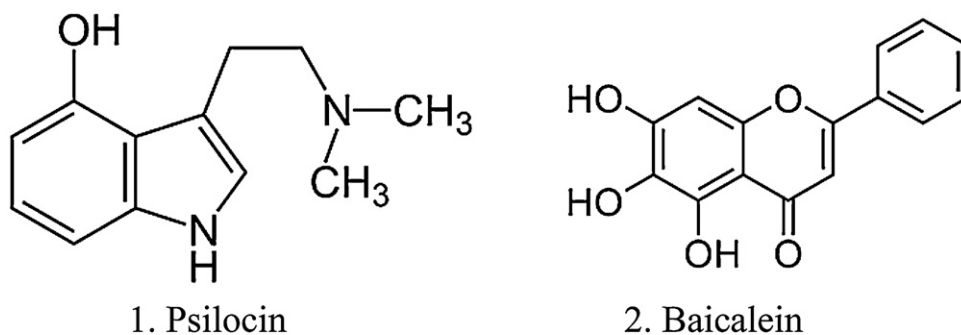
Fig. 1. Chemical structures of psilocin and baicalein.

from Sigma. Ammonium acetate ($AcONH_4$) was supplied by Merck (Darmstadt, Germany). *G. spectabilis* was provided by the Institute of Mycology, Jilin Agricultural University, and identified by Dr. Tolgor Bau at the institute; a voucher specimen was deposited at the College of Traditional Chinese Medicinal Material, Jilin Agricultural University.

2.2. Animal handling

Sprague-Dawley rats (220–250 g) were obtained from the Animal Center of the Chinese Academy of Sciences and housed with unlimited access to food and water, except while fasting 12 h before the experiment. The animals were maintained on a 12-h light/12-h dark cycle (lights on at 08:00) at ambient temperature (22–25 °C) and 60% relative humidity.

2.3. Chromatography

UPLC was performed on an Acquity UPLC system (Waters, Milford, MA, USA) equipped with a binary solvent delivery system, an autosampler, and a PDA detection system. Gradient elution was carried out at a constant flow rate of 0.5 mL/min. The mobile phase consisted of 50 mM $AcONH_4$ in water (A) and acetonitrile (B). The separation was performed on an ODS C_{18} column (2.3 μm, 100 mm × 2.1 mm I.D.; Tosoh, Tokyo, Japan) at 25 °C. Initial mobile phase condition was 10% B and increased to 25% B for 2 min, increased to 55% B for 3 min, then maintained for another 5 min. The monitoring wavelength was set at 265 nm.

2.4. Sample preparation

First, 20 g of dried *G. spectabilis* was crushed to powder and then 100 mL water was added to the powder. The solution was sonicated for 1 h at a temperature lower than 50 °C; the solution was obtained by filtration and the residues were extracted two times using the same method. The combined aqueous solution was evaporated to about 20 mL under reduced pressure, and the psilocin concentration was determined by UPLC-PDA. The decoction was stored in a refrigerator at 4 °C before oral administration to rats.

Plasma samples were prepared using the following steps: 100 μL plasma was obtained through the centrifugation of orbital blood; then, the plasma was spiked with 10 μL of baicalein (100 μg/mL in methanol) as an internal standard (IS) and 900 μL acetonitrile was added to remove protein. After centrifuging, the supernatant acetonitrile was withdrawn and the sample dried by nitrogen gas. The residue was dissolved in 100 μL methanol and stored at −20 °C for further analysis. Data from these samples were used to construct the pharmacokinetic profiles by plotting drug concentration vs. time curves.

2.5. Psilocin content of the G. spectabilis extract

The psilocin concentration was determined using UPLC-PDA developed and validated in our laboratory. Approximately 100 μL of extract was diluted fivefold in water and vortexed for 1 min. Then, the diluted solution was centrifuged at 18,000 rpm for 10 min and filtered through a 0.2-μm microporous membrane before injection. The psilocin content in the *G. spectabilis* extract was 1.01 mg/mL.

2.6. Calibration standard and quality control samples

The psilocin reference standard and IS were weighed accurately and dissolved in methanol at concentrations of 1 mg/mL and stored at −20 °C. Then, they were diluted to the appropriate concentration ranges to construct calibration curves in rat plasma. Psilocin reference standard solutions at six different concentrations (0.2, 0.5, 1.0, 5, 10, and 20 μg/mL) containing 10 μg/mL IS were prepared by spiking 100 μL of plasma with appropriate volumes of the standard stock solution.

High-, mid-, and low-level quality control (QC) samples containing 0.5, 5, and 20 μg/mL of the reference standard, respectively, were prepared in a manner similar to that used to prepare the calibrator samples.

2.7. Precision and accuracy

The three concentrations (high, medium, and low) of standard solution in rat plasma were detected five times on the same day to determine the intraday variability and on 5 consecutive days to determine the inter-day variability. The precision coefficient of variation (RSD) was calculated as %RSD = [standard deviation (S.D.)/C_{obs}] × 100. The accuracy was calculated as % = [C_{obs}/C_{the}] × 100, where C_{obs} is the observed concentration and C_{the} is the theoretical concentration. The limits of detection (LOD) and limits of quantification (LOQ) for the standard solutions were calculated with a signal-to-noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively.

2.8. Recovery and stability

The recovery of psilocin was determined by comparing the peak areas between plasma samples spiked with psilocin (0.5, 5, and 20 μg/mL) and methanol spiked with psilocin at the corresponding concentrations.

The stability of the psilocin in plasma was determined using five replicates of QC samples at each QC level using four different sample preparatory methods (freeze–thaw, long-term, short-term, and post-preparative plasma samples). In each freeze–thaw cycle, the QC samples were frozen at −20 °C for about 24 h and thawed at room temperature (20 °C). The long-term stability was evaluated

Table 1
Precision and accuracy of psilocin in rat plasma.

Spiked (µg/mL)	Intra-day ($n=5$)			Inter-day ($n=5$)		
	Measured (µg/mL)	Accuracy (%)	Precision (RSD%)	Measured (µg/mL)	Accuracy (%)	Precision (RSD%)
0.5	0.49	98.0	2.6	0.46	92.0	3.5
5	4.8	96.0	5.7	5.1	102.0	5.1
20	20.5	102.5	5.1	20.7	103.5	6.3

Table 2
The stability of psilocin in rat plasma under different conditions.

	Accuracy (mean ± S.D.%)		
	0.5 µg/mL	5 µg/mL	20 µg/mL
Short-term stability	102.2 ± 5.1	91.7 ± 1.5	95.5 ± 2.1
Long-term stability	97.6 ± 6.4	96.9 ± 2.9	102.6 ± 4.9
Freeze–thaw stability	98.3 ± 6.7	97.3 ± 2.8	95.4 ± 1.5
Post-preparative stability	102.4 ± 2.4	104.2 ± 3.8	103.7 ± 4.6

after keeping the QC samples frozen at −20 °C for 14 days. For short-term stability, frozen samples were kept at room temperature for 5 h before sample preparation. The post-preparative stability of the processed samples was tested after keeping the samples in HPLC autosampler vials at room temperature for 24 h.

2.9. Pharmacokinetic study

Experiments were performed on ten rats. The rats were given the *G. spectabilis* extract orally by gavage with a gauge syringe (1 mL/100 g body weight). Blood samples (each ca. 0.5 mL) were collected via the orbital vein at specific times (before administration and 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, and 420 min after administration). This study was approved by an Ethic Committee, Animal Lab, College of Traditional Chinese Medicinal Material, Jilin Agricultural University, China. The blood samples were centrifuged at 17,000 rpm for 10 min at 4 °C. Then, 100-µL plasma samples were withdrawn and mixed with 900 µL of acetonitrile to remove any protein. After centrifuging, the supernatant acetonitrile was withdrawn and the residue dried by nitrogen gas. The residue was dissolved in 100 µL of methanol and stored at −20 °C until further analysis. Data from these samples were used to construct the pharmacokinetic profiles by plotting drug concentration vs. time curves. All the data were processed using non-compartmental

analysis using the Drug and Statistics (DAS) 2.0 package (Chinese Pharmacological Society).

3. Results and discussion

3.1. Developing the UPLC method

Different mobile phases have been used to separate psilocin from related endogenous substances. We also investigated the use of several mobile phases to optimize the method. The mobile phase consisted of 50 mM $AcONH_4$ in water (A) and acetonitrile (B). The separation was performed on an ODS C_{18} column at a column temperature of 25 °C. The monitoring wavelength was set at 265 nm, because at this wavelength psilocin produced a better signal to noise response. At the selected UPLC conditions psilocin was base line separated from other matrices.

3.2. Method validation

3.2.1. Selectivity
The sample formed a clear peak in endogenous plasma samples; the IS peak was separated from the psilocin peak without any interference. The average elution times of psilocin and IS were 2.9 and 4.6 min, respectively. Fig. 2 shows representative UPLC-PDA chromatograms of (A) blank plasma, (B) plasma spiked with psilocin and IS, (C) plasma obtained 120 min after the oral administration of *G. spectabilis*, and (D) plasma spiked with psilocin at LOQ.

3.2.2. The linearity of the calibration curve
Plasma samples spiked with different concentrations of standard solution were processed as described in Section 2. The calibration curves were constructed by calculating the peak area ratios for standard concentrations. The calibration curves were linear within the concentration range assayed. The mean value of the regression equation of the analyte in rat plasma was $y = 0.45x + 1.47$
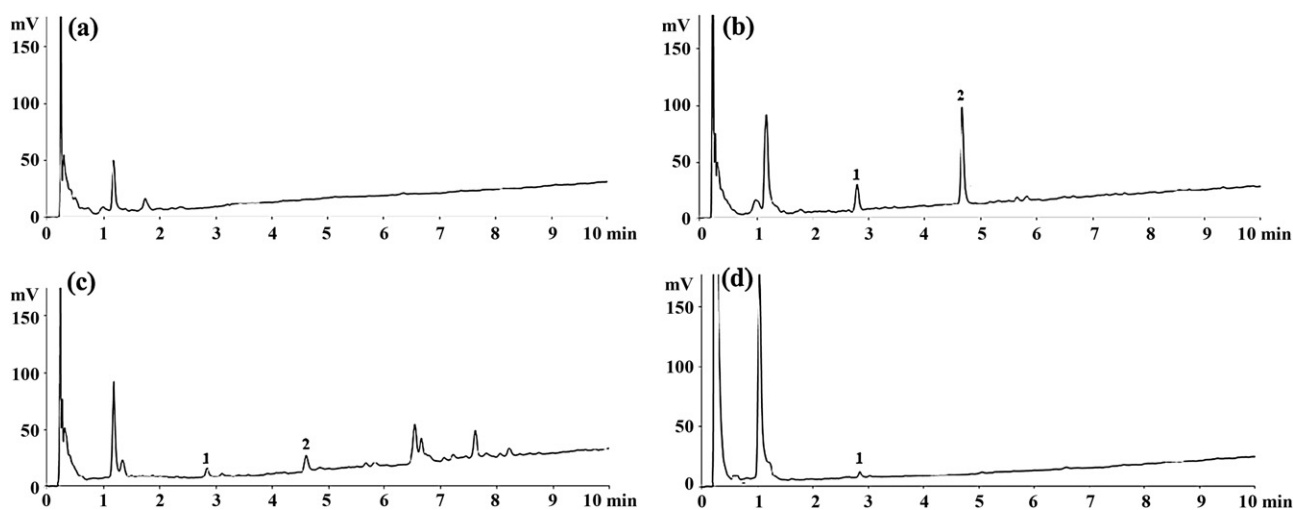

Fig. 2. The representative UPLC-PAD chromatograms (a) blank plasma, (b) blank plasma spiked with psilocin and baicalein standard, (c) plasma sample obtained 120 min after oral administration of *Gymnopilus spectabilis*, (d) blank plasma sample spiked with psilocin at the level of LOQ. Peak identification: 1. Psilocin, 2. Baicalein.

Table 3
Pharmacokinetic parameters of psilocin after oral administration of *Gymnopilus spectabilis* extract.

Parameters	C_{max} (μg/mL)	T_{max} (min)	AUC$_{0\to t}$ ((μg min)/mL)	AUC$_{0\to\infty}$ ((μg min)/mL)	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	K_{12} (min^{-1})	K_{21} (min^{-1})	K_{10} (min^{-1})	V1/F (mL)	CL/F (min^{-1})
Value	0.43 ± 0.12	90 ± 2.1	1238.3 ± 96.4	2231.2 ± 101.2	117.3 ± 40.3	148.5 ± 62.6	1.3 ± 0.41	1.9 ± 0.71	2.1 ± 0.83	3.2 ± 1.7	2.2 ± 0.9

C_{max}, the maximum plasma concentration; T_{max}, the time to reach C_{max}; AUC, the area under the plasma concentration–time curve; $T_{1/2\alpha}$, distribution half-life (the half-life of the α phase); $T_{1/2\beta}$, elimination half-life (the half-life of the β phase); K_{12}, distribution rate constant for transferring the drug from the central to peripheral compartment; K_{21}, transfer rate constant from peripheral to central compartment; K_{10}, elimination rate constant; V1/F, apparent central volume of distribution; CL/F, the apparent oral elimination clearance.
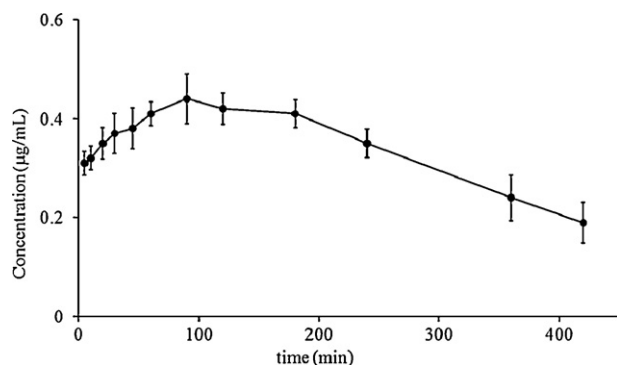

Fig. 3. The plasma concentration with standard deviation vs. time profile of psilocin after oral administration (about 1.01 mg psilocin/100 g body weight) of *Gymnopilus spectabilis* extract.

($r^2 = 0.9992$). The LOQ of psilocin was 0.12 μg/mL and the LOD was 0.05 μg/mL in plasma.

3.2.3. Accuracy and precision

The precision and accuracy of the assay were estimated by analyzing QC samples at low, middle, and high concentrations. The concentrations of the QC samples were calculated from the calibration curve performed on the same day. The results are shown in Table 1. The intraday precision (RSD) ranged from 2.6 to 5.7% and the inter-day precision (RSD) from 3.5 to 6.3%. The intraday accuracy ranged from 96.0 to 102.5% and the inter-day accuracy from 92.0 to 103.5%.

3.2.4. Recovery and stability

The recovery levels of psilocin at each QC level (0.5, 5, 20 μg/mL) were 98.9 ± 1.6%, 104.8 ± 1.4%, and 97.7 ± 2.4%, respectively. The stability of the psilocin in processed samples, after freeze–thaw cycles and after long-term cold storage was evaluated and is summarized in Table 2. The results suggest that the psilocin is stable for 24 h after preparation, for 14 days under cold storage, and over three freeze–thaw cycles in plasma samples, since no obvious change in the psilocin concentrations was observed in plasma tested within the time period under the indicated storage conditions.

3.3. Pharmacokinetic study of psilocin

The established method was applied to the analysis of plasma samples after the oral administration of a *G. spectabilis* extract. The mean plasma concentration–time profile of psilocin is shown in Fig. 3, and the relevant pharmacokinetic parameters are listed in Table 2. The results differed from previous reports [9,23,24], perhaps because previous studies examined the oral or intravenous administration of pure psilocin, while the *G. spectabilis* extract contains other chemicals. Herb–drug interactions changed the pharmacokinetic parameters of psilocin in the rats. Another reason might be the use of different animals and biological samples. After the oral administration of the *G. spectabilis* extract,

psilocin was rapidly absorbed into blood and reached a peak concentration of 0.43 ± 0.12 μg/mL at 90 ± 2.1 min. After peaking, the psilocin plasma concentration decreased with a mean $T_{1/2\beta}$ of 148.5 ± 62.6 min; the LOQ of the assay was reached within 420 min. The average AUC$_{0\to\infty}$ was 2231.2 ± 101.2 (μg/mL) min. A mean apparent oral elimination clearance (CL/F) of 2.2 ± 0.9 l/min and a mean apparent central volume of distribution (V1/F) of 3.2 ± 1.7 mL were calculated. The detailed pharmacokinetics of psilocin in rat plasma is given in Table 3.

4. Conclusion

To investigate the pharmacokinetics of psilocin, the main psychoactive compound of *G. spectabilis*, UPLC-PDA was developed for reliable quantitative determination of psilocin in rat plasma. Our method is simple, useful, and sensitive for assaying psilocin in rat plasma after the oral administration of *G. spectabilis* extract. The method shows good recovery, accuracy, and precision, indicating that it is sufficiently valid to meet the requirements for the pharmacokinetic study of *G. spectabilis*.

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