SHORT COMMUNICATION

Sensitive quantitative analysis of psilocin and psilocybin in hair samples from suspected users and their distribution in seized hallucinogenic mushrooms

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Received: 28 October 2020 / Accepted: 17 December 2020 / Published online: 2 February 2021 © The Author(s) 2021

Abstract
Purpose In this study, we developed a very sensitive method for quantitative analysis of psilocin and psilocybin in hair samples of magic mushroom consumers.

Methods The analyses were performed with pretreatments of samples, followed by ultra-high pressure liquid chromatography (LC) connected to a Q-Trap type tandem mass spectrometry (MS/MS). For LC, mobile phase (A) consisted of 0.1% formic acid in water, and mobile phase (B) was acetonitrile for gradient elution using a Acquity™ UPLC HSS T3 column. For MS/MS, electrospray ionization measurements in positive selected reaction monitoring mode were used.

Results The calibration curves were linear from 5 to 500 pg/mg ($r > 0.99$) and no selectivity problems occurred. The limit of detection was 1 pg/mg, and the lower limit of quantitation was 5 pg/mg. The ranges of the matrix effects and recovery rates were 90.4–107% and 76.0–102%, respectively.

Conclusions The concentrations of psilocin in two authentic hair were 161 and 150 pg/mg, respectively, and psilocybin was not detected from both samples. This method was also used to analyze the distribution of psilocin and psilocybin in seven hallucinogenic mushrooms. To our knowledge, this is the first demonstration of psilocin concentrations in hair samples of hallucinogenic mushroom consumers, and also our method is most sensitive for quantitative analysis of psilocin and psilocybin in hair samples.

Keywords Psilocin · Psilocybin · Hair analysis · Hallucinogenic mushrooms · UHPLC–MS/MS

Introduction
Psilocin and psilocybin are the main hallucinogenic compounds of mushrooms such as Psilocybe, Panaeolus and Conocybe mushrooms that are commonly known as “magic mushrooms” or “hallucinogenic mushrooms”. These compounds belong to the new psychoactive substance class of tryptamines and are traditional natural neuropsychiatric drugs that can cause hallucinogenic effects after taking them, producing time and space deformation and hallucinations. Hallucinogenic mushrooms were first used by North American Indians in religious ceremonies, and they were gradually abused because they can make people feel happy [1, 2]. In recent years, cases of the abuse of hallucinogenic mushrooms have been reported in many countries. Abusers usually abuse the dried products of hallucinogenic mushrooms, or grind the hallucinogenic mushrooms.
mushrooms into powder and consume the powder in the capsule form. Additionally, psilocin and psilocybin were listed in Category I of the Control Catalogue of Psychotropic Drugs of China in 2013. Long-term use of such hallucinogenic mushrooms will cause people to have adverse mental symptoms, even addiction, and these individuals will be unable to extricate themselves [3, 4].

Since psilocin is easily oxidized during sample preparation and/or psilocybin is dephosphorylated into psilocin in the human body by alkaline phosphatase, it is quite difficult to accurately and reliably determine trace amounts of psilocin and psilocybin in biological samples [4]. Several methods have been developed for the identification of psilocin and psilocybin in hallucinogenic mushroom, urine, and blood samples [5–11]. Many forensic toxicology laboratories use gas chromatography (GC) or GC–mass spectrometry (MS) to detect psilocin and psilocybin in hallucinogenic mushroom [12], blood [13] or urine [14] samples after derivatization. High-performance liquid chromatography (HPLC)–ultraviolet (UV) detection has also been used to analyse psilocin and psilocybin in hallucinogenic mushrooms [7, 15] and plasma [9]. In addition, some validated liquid chromatography (LC)–MS/MS methods were used to analyse psilocin and psilocybin in hallucinogenic mushroom [16], urine [17] and blood [6, 11] samples. Given that the metabolism of psilocin is rapid and unstable under the influence of light and air, the analysis of psilocin in body fluids is relatively challenging [18]. There is only one report describing the quantification of psilocybin (0.8 ng/mg) in the hair of a *Psilocybe* consumer [19]. However, there are no reports on the analysis of psilocin in authentic human hair.

This study proposes a sensitive and highly specific ultra-high-performance liquid chromatography (UHPLC)–MS/MS method for quantitative analysis of psilocin and psilocybin in seized hallucinogenic mushrooms and hair from suspected users (Fig. 1).

### Materials and methods

#### Chemicals and reagents

Psilocin (1 mg/mL), psilocybin (1 mg/mL), and the internal standards (ISs) psilocin-d$_{10}$ and psilocybin-d$_{4}$ (100 μg/mL) were purchased from Cerilliant (Round Rock, TX, USA); HPLC-grade methanol and acetonitrile from Sigma-Aldrich (St. Louis, MO, USA); analytical-grade acetone from Shanghai Lingfeng Chemical Reagent Co. (Shanghai, China); formic acid (98%) from Fluka (Buchs, Switzerland); and deionized water was produced by an in-house Milli-Q water system (Millipore, Burlington, MA, USA).

#### In vitro and in vivo samples

The seven suspicious mushrooms used to identify psilocin and psilocybin were obtained from the police and customs with permission.

Human blank (drug-free) hair came from colleagues who had no history of drug use [20]. Authentic hair samples used to analyse psilocin and psilocybin were obtained from suspected users. All hair samples were collected with written informed consent.

#### Preparation of calibrators and quality control sample

A mixed stock solution containing psilocin and psilocybin was diluted with methanol to reach each concentration of 10 μg/mL. From this solution, working solutions with concentrations at 1000, 100 and 10 ng/mL in methanol were prepared. The IS stock solution was prepared by mixing 100 μL of 100 μg/mL psilocin-d$_{10}$ or psilocybin-d$_{4}$ in methanol. The IS stock solution was diluted with methanol to provide an IS working solution at a concentration of 100 ng/mL.

Calibration standards were prepared by spiking blank hair with the working solutions to obtain final concentrations of 5, 10, 50, 100, 200, 400 and 500 pg/mg for psilocin and psilocybin. Low (5 and 50 pg/mg), medium (100 pg/mg) and high (400 pg/mg) concentrations were used for quality control (QC) samples. All standard stock solutions, working solutions, and QC samples were stored at −20 °C.

#### Solid sample preparation

An appropriate amount of the suspicious mushroom was cut with scissors. Twenty milligrams of each mushroom was weighed and placed in a 2 mL grinding tube, and 1 mL of extraction solvent (methanol) was added. The sample was placed in a Shanghai Jing Xin JXFSPRP-CLN freeze
grinding machine (Shanghai Jing Xin Industrial Development Co., Ltd., Shanghai, China), and it was ground twice at a temperature below 4 °C. The parameters were set as follows: speed, 2500 rpm; number of runs, 15 times; interval time, 60 s; and grinding, 18 m/s. Then, the sample was centrifuged at 13,500 × g for 5 min. A total of 200 μL of the supernatant was placed directly into an autosampler vial for GC–MS analysis. Subsequently, the supernatant was diluted 10,000 times with 0.1% formic acid in water and directly analysed by LC–MS/MS.

Hair samples were consecutively rinsed three times with acetone. After drying at room temperature, the hair was cut into 1–2 mm pieces with scissors. Subsequently, 20 mg of hair was weighed and placed in a 2 mL grinding tube, and 0.5 mL of extraction solvent (composed of 0.1% formic acid-water containing 1 ng of ISs) was added. Then, the sample was placed in the Shanghai Jing Xin JXFSPRP-CLN freeze grinding machine for grinding under the same condition as that for mushroom. The sample was centrifuged twice at 14,100 × g for 3 min each time. Finally, 200 μL of the supernatant was pipetted into the autosampler vial, and 5 μL was injected into the LC–MS/MS.

**GC–MS**

GC–MS analysis was performed using an Agilent 7890B GC/5977A MS (Agilent Technologies, Santa Clara, CA, USA). An Agilent 7890B gas chromatograph equipped with an HP-5MS analytical column (30 m × 0.25 mm, film thickness 0.25 mm; Agilent Technologies) and helium as the carrier gas (1.0 mL/min) was employed. The GC was coupled to an HP5977A mass detector in electron ionization (EI) mode at 70 eV. The temperature was held at 100 °C for 1.5 min, increased at 25 °C/min to 280 °C, and then held for 15 min. The ion source and quadrupole temperatures were 230 and 150 °C, respectively. The inlet and interface temperatures were 250 and 280 °C, respectively. The scanning range was m/z 40–400 at 0–3 min, and the scanning range was m/z 50–550 at 4–6 min.

**LC–MS/MS**

LC–MS/MS analysis was performed using an ultra-high pressure Acquity™ Ultra Performance LC system (Waters Corporation, Milford, MA, USA) and a Sciex 6500 Plus Q-trap™ quadrupole mass spectrometer fitted with a Turbolon Spray interface (AB Sciex, Framingham, MA, USA). The data were analysed using MultiQuant 3.0.2 software (AB Sciex).

The analytes were separated on a Waters Acquity TM UPLC HSS T3 column (100 × 2.1 mm i.d., partial size 1.8 μm) using linear gradient elution. Mobile phase A consisted of 0.1% formic acid in water. Mobile phase B was acetonitrile. The gradient was programmed as shown in Table 1. The flow rate was set to 0.2 mL/min. The total running time was 8 min, and the injection volume was 5 μL. The temperature of the autosampler was maintained at 4 °C.

The mass spectrometer was operated with positive electrospray ionization and multiple reaction monitoring (MRM) mode. The mass spectrometric parameters were set as follows: ion source temperature, 500 °C; curtain gas, 20 psi (nitrogen); collision cell exit potential, 9 V; ion source gas 1, 30 psi; ion source gas 2, 30 psi; entrance potential, 10 V; ion spray voltage, 5000 V; and collision activation dissociation gas, low. The MRM transitions and retention times are shown in Table 2.

**Method validation**

The method was validated according to the methodology validation guidelines [21, 22], including selectivity, linearity, limit of detection (LOD), lower limit of quantification (LLOQ), accuracy, precision, matrix effect and recovery.
Selectivity

The selectivity was evaluated by analyzing hair samples from 10 different sources for the potential endogenous compounds in the hair or ISs interfering with the detection of analytes.

Linearity, LOD, and LLOQ

Calibrators were prepared at concentrations of 5, 10, 50, 100, 200, 400 and 500 pg/mg for psilocin and psilocybin. Linearity was determined by analyzing and processing seven sets of calibrators and generating calibration curves with 1/x weighting, and two independent analyses were performed for each set. The LOD and LLOQ were evaluated by spiked blank hair samples with decreasing concentrations of the analytes. The LOD was determined as the lowest analyte concentration with a signal-to-noise (S/N) ratio of at least 3. The LLOQ was defined as the lowest concentration of linearity with an S/N ≥ 10 and that had acceptable precision and accuracy.

Accuracy and precision

Accuracy and precision were studied for hair samples by measuring six replicates at the LLOQ and QC samples at 5, 10, 100 and 400 pg/mg. The intraday and interday precisions were evaluated by the percentage coefficient of variation (% CV). The accuracy was assessed based on the percentage ratio of the measured nominal concentration (mean of measured/nominal × 100). The precision should not exceed ± 15% for the low, medium and high concentrations, except for the LLOQ, which should not exceed ± 20%. The accuracy should be within the range of 85–115% for the low, medium and high concentrations, except for the LLOQ, which should not be out of range of 80–120%.

Matrix effect and recovery

The matrix effect and recovery were evaluated according to the method proposed by Matuszewski et al. [23] at 10, 100, and 400 pg/mg with 6 replicates. The samples are divided into three groups: pre-extraction spiked samples, post-extraction spiked samples and standard solutions in 0.1% formic acid in water (neat sample). The matrix effect value is the peak area of post-extraction spiked samples divided by the peak area of the standard solution in 0.1% formic acid in water. The recovery is calculated as the peak area of pre-extraction-spiked samples divided by the peak area of post-extraction-spiked samples.

Results

Selection of the instrument

In our forensic toxicology laboratory, in vitro samples are usually analysed by GC–MS technology. We used GC–MS technology to analyse psilocin and psilocybin in hallucinogenic mushrooms and found that both psilocin and psilocybin had chromatographic peaks at 8.454 and 8.455 min, respectively (Fig. 2). Then, the GC–MS technique was used to analyze the 10 μg/mL standard solutions of psilocin and psilocybin, respectively, and the chromatographic peaks appearing at 8.454 min. The analysis showed that the chromatographic peak appeared at 8.454 min was only psilocin. According to relevant literature reports, psilocybin was prone to thermal decomposition and decarboxylation to form psilocin without derivatization under GC conditions [12–14]. Therefore, the GC–MS method cannot distinguish between psilocin and psilocybin without derivatization. In addition, LC–MS and LC–MS/MS have the advantages of analyzing compounds that are easily decomposed by heat and are inherently highly sensitive [24]. Therefore, we chose to use LC–MS/MS technology, which did not require derivatization to analyse psilocin and psilocybin in hallucinogenic mushrooms. The chromatographic separation conditions were optimized to obtain good resolution and peak shape. After optimization, 0.1% formic acid in water (A) and acetonitrile (B) were selected as the mobile phase; good resolution and good peak shape were obtained, and a gradient elution procedure was adopted.

Optimization of sample preparation

Psilocin and psilocybin are unstable in solution, and they are completely inactivated after storage in methanol at room temperature for several months. According to relevant literature reports, psilocin and psilocybin were stable in acidic solutions [17]. There have been some reports in the literature about the use of acidic solvents to extract tryptamine compounds [25]. Therefore, we optimized four extraction solvents: 1% formic acid-methanol, 1% formic acid-water, 0.5% formic acid-water, and 0.1% formic acid-water. When methanol was used as the extraction solvent, tailing peaks tended to appear in the chromatogram. By comparing the extraction recovery and matrix effect of different extraction solvents, the results showed that 0.1% formic acid-water was the best extraction solvent for hair samples. Moreover, Martin et al. [25] used hydrochloric acid-methanol extraction and sonication for 6 h to extract psilocin from 50 mg of hair and performed extract cleanup by solid-phase extraction using a mixed-mode cation
We used a Shanghai Jing Xin JXFSPRP-CLN freeze grinding machine to grind approximately 20 mg of human hair below 4 °C and utilized 0.1% formic acid–water to extract psilocin and psilocybin. The cryo-grinding method ensured the stability of thermally unstable compounds, and at the same time, it could pulverize the hair completely so that the drug in the hair was released relatively completely, and the operation was simple and timesaving. In addition, the method developed in this study provided higher sensitivity and a lower limit of quantification than those of the previous method [6, 11, 16, 17].

**Method validation**

The selectivity of the method was evaluated using 10 blank hair matrix samples and internal standards from different sources, and no endogenous interference peaks or interference from the ISs with the analytes were observed.

The linearity, LOD, and LLOQ of psilocin and psilocybin in human hair are listed in Table 3. The linear range was 5–500 pg/mg, and the correlation coefficient ($r$) was higher than 0.99. The LOD value of psilocin and psilocybin was 1 pg/mg, and the LLOQ value was 5 pg/mg for human hair samples. The LOD and LLOQ values of this method were lower than those of previous studies (LOD = 10 pg/mg, LLOQ = 22 pg/mg) [25], which means that this method has sensitivity higher than any analytical method for psilocin and psilocybin in human hair.

The accuracy and intraday and interday precisions of psilocin and psilocybin at the four levels are shown in Table 4. The accuracy and intraday and interday precision values met the acceptable criteria. The intraday precisions ranged from 6.1 to 10.5%, and the interday precisions ranged from 2.2 to 6.7%. The accuracy was between 93.6 and 112%.

The results of the matrix effect and recovery analyses are also shown in Table 4. The matrix effects of psilocin and psilocybin ranged from 90.4 to 107% at 10 (low), 100 (medium), and 400 pg/mg (high) (% RSD ≤ 20%). The results indicated that the analytes did not exhibit significant ion suppression or ion enhancement in this method. The recoveries of psilocin and psilocybin were within a range of 76.0–102%.

**Table 3** Linearity, limit of detection (LOD) and lower limit of quantification (LLOQ) for psilocin and psilocybin in human hair obtained by the UHPLC–MS/MS

| Analyte    | Linearity (pg/mg) | Regression equation | Correlation coefficient ($r$) | LOD (pg/mg) | LLOQ (pg/mg) |
|-----------|-------------------|---------------------|-------------------------------|-------------|--------------|
| Psilocin   | 5–500             | $y = 0.3652x - 0.0003161$ | 0.994                         | 1           | 5            |
| Psilocybin | 5–500             | $y = 1.99x - 0.00371$  | 0.992                         | 1           | 5            |
Application of the method

The UHPLC–MS/MS method proposed in this study was used to successfully detect and quantitatively analyze psilocin and psilocybin in seized hallucinogenic mushrooms and two authentic human hair samples.

Quantitative analysis of psilocin and psilocybin in seized hallucinogenic mushrooms

In recent years, hallucinogenic mushrooms have often been sold in their dried form, ground powder or capsules. At present, there have been some reports in the literature using LC–MS or GC–MS to analyse the content and distribution of psilocin and psilocybin in hallucinogenic mushrooms [15]. The psilocin content in six hallucinogenic mushrooms was determined to be lower than 12.67 ng/mg by HPLC combined with the fluorescence derivatization method [4]. The police seized a batch of suspicious mushrooms during a customs security check and sent them to our forensic toxicology laboratory for testing (Fig. 3). Seven suspicious mushrooms and 50 ng/mL mixed standard dilutions of psilocin and psilocybin were separately analysed by LC–MS/MS. Both the suspicious mushroom and the standard samples showed chromatographic peaks at 4.04 min and 2.29 min, indicating that the suspicious mushroom contained psilocin and psilocybin (Fig. 4). The quantitative analysis results of psilocin and psilocybin in 7 suspicious mushrooms are shown in Table 5. The results showed that the contents of psilocin and psilocybin in the canopy of hallucinogenic mushrooms were higher than those in the stem (Table 5).

Quantitative analysis of psilocin and psilocybin in hair samples of suspected users

A 25-year-old man (case 1) was driving home, speeding, and laughing. The driver was stopped by the police, and the remaining suspicious poisonous mushrooms were seized from the car. It was found that the driver’s ability to respond was low; his eyes were dull and red, and his standing was unstable. He was brought to the hospital immediately and recovered. The concentration of psilocin detected in his hair was 161 pg/mg, and psilocybin was not detected.

A 31-year-old male (case 2) with a history of drug use went to a party at an entertainment venue. At the party, he drank and smoked suspicious powder. After 30 min, he experienced hallucinations, felt his surroundings moved, and laughed. After receiving the report, the police seized the suspicious powder at the scene and sent the man to the hospital. The suspicious powder contained psilocin and psilocybin. The concentration of psilocin detected in his hair was 150 pg/mg, and psilocybin was not detected (Fig. 5).

Discussion

It has been reported that in the human body, psilocybin is rapidly dephosphorylated to form psilocin, and most psilocin is combined with glucuronic acid and excreted in urine. Small amounts of psilocin and psilocybin in hallucinogenic mushrooms can induce ecstasy, but overdose or administration with additional drugs can be fatal [9].

Table 4 Accuracies, precisions, matrix effects and recoveries for psilocin and psilocybin in human hair measured by the UHPLC–MS/MS

| Analyte | Concentration (pg/mg) | Accuracy (%) | Precision (%) | Matrix effect (%) | Recovery (%) |
|---------|-----------------------|--------------|---------------|------------------|--------------|
|         |                       | Intraday (n = 6) | Interday (n = 6) | (n = 6)          | (n = 6)      |
| Psilocin | 5                     | 108          | 6.1           | 2.2              | –            | –            |
|         | 10                    | 96.4         | 10.5          | 6.7              | 93.1         | 87.1         |
|         | 100                   | 97.5         | 9.1           | 3.9              | 90.8         | 76.0         |
|         | 400                   | 106          | 9.2           | 5.5              | 103          | 102          |
| Psilocybin | 5                     | 112          | 9.5           | 2.8              | –            | –            |
|         | 10                    | 93.6         | 8.3           | 4.2              | 90.4         | 88.2         |
|         | 100                   | 94.6         | 9.3           | 4.0              | 107          | 92.0         |
|         | 400                   | 110          | 9.8           | 4.0              | 104          | 101          |

Fig. 3 An example of the seized hallucinogenic mushrooms containing psilocin and psilocybin
Sticht and Käferstein [13] used GC–MS and silylation to detect psilocin in urine and serum and found that psilocin mostly existed in the conjugated form, and the concentration of psilocin detected in urine and blood was 0.018 and 0.052 mg/L, respectively. However, Tiscione and Miller [26] analysed the concentration of free psilocin in urine samples to be 0.23 mg/L by GC–MS. The analysis of psilocin in body fluids is relatively challenging because psilocin is rapidly metabolized and unstable under the influence of light and air [18].

As to psilocin concentration in human hair samples collected from magic mushroom consumers, no report on its detection appeared. Only one report on the quantitative detection of psilocybin from hair (0.8 ng/mg) of magic mushroom consumer appeared, but psilocin was not detected [19]. Therefore, this report is the first to succeed

![Fig. 4](image_url)  
Multiple reaction monitoring (MRM) chromatogram for psilocin and psilocybin in the canopy and stem

| Case | Mushroom canopy | Ratio | Stem | Ratio |
|------|----------------|-------|------|-------|
|      | Psilocybin (ng/mg) | Psilocin (ng/mg) | Psilocybin (ng/mg) | Psilocin (ng/mg) |
| 1    | 5.49            | 6.24  | 0.88 | 4.11  | 5.95  | 0.69  |
| 2    | 4.77            | 5.39  | 0.88 | 1.18  | 2.70  | 0.44  |
| 3    | 1.32            | 9.59  | 0.14 | 1.50  | 4.39  | 0.34  |
| 4    | 1.22            | 3.03  | 0.40 | 0.755 | 1.89  | 0.40  |
| 5    | 1.42            | 14.9  | 0.10 | 0.164 | 0.883 | 0.19  |
| 6    | 1.22            | 7.50  | 0.16 | 0.033 | 0.837 | 0.04  |
| 7    | 0.62            | 19.6  | 0.03 | 0.296 | 3.28  | 0.09  |
quantitative detection of psilocin from authentic human hair.

The reason why the detection of psilocin in hair specimens of magic mushrooms should be pointed out. First, we need the most modern Q-trap type MS instrument, which seems to be extraordinarily sensitive, coupled to a special high-quality UHPLC system. Second, the fresh hair specimens were cut off just before the beginning the analysis, followed by pulverizing to fine powder. Third, special care was taken to prevent the specimens from the decomposition of psilocin by light [17] at low temperature, using alkaline pH solution during the pretreatment. As results, the concentrations of psilocin in hair specimens from two subjects were 161 and 150 pg/mg, respectively. Psilocybin could not be detected from both hair specimens, despite that the LOD and LLOQ were as low as 1 and 5 pg/mg, respectively, which seems due to metabolism of psilocybin to psilocin in vivo in the humans.

At the next study, we have studied the distribution of psilocin and psilocybin in the canopies, stems and hyphae of hallucinogenic mushrooms themselves. Since such studies had been well conducted [12, 15, 16], we compared our results with the published data as shown in Tables 5 and 6. Our results and those in the previous studies [7, 12] equally indicated that the concentration of psilocybin in the stems and hyphae of hallucinogenic mushrooms are lower than in the canopy. We examined the ratio of psilocybin to psilocin in the canopies and stems of hallucinogenic mushrooms, and the results are listed in Table 5. The ratio of psilocybin to psilocin in the canopies of the 7 samples ranged from 0.03 to 0.88, while the ratio of psilocybin to psilocin in the stems ranged from 0.04 to 0.69. The results show that the psilocin content is higher than the psilocybin content in the canopies and stems of hallucinogenic mushrooms. The data in the previously published literature showed that the contents of psilocybin in the canopies and stems of hallucinogenic mushrooms were higher than those of psilocin, which was exactly the opposite of our experimental results [12, 15, 16]. However, the literature published by Tsujikawa et al. [27]

Table 6 Data on the contents of psilocin and psilocybin in hallucinogenic mushrooms reported in the relevant literatures

| Case | Species                        | Psilocybin (ng/mg) | Psilocin (ng/mg) | Ratio | References |
|------|--------------------------------|--------------------|-----------------|-------|------------|
| 1    | *Psilocybe sergenteipes*       | 3.8                | 0.69            | 5.51  | [16]       |
| 2    | *Psilocybe sergenteipes*       | 3.2                | 0.60            | 5.33  |            |
| 3    | *Psilocybe subcubensis*        | 1.5                | 1.0             | 1.5   |            |
| 4    | Unknown                        | 0.18               | 1.4             | 0.13  |            |
| 5    | *Psilocybe cubensis* (dried)   | 11.51              | 1.26            | 9.17  |            |
| 6    | *Psilocybe cubensis* (fresh)   | 7.92               | 0.66            | 12.00 |            |
| 7    | *Psilocybe cubensis* (grow box)| 5.09               | 1.27            | 4.01  |            |
| 8    | *Panaeolus cyanescens* (dried)| 25.14              | 11.94           | 2.10  |            |
| 9    | *Psilocybe tampanensis* (fresh)| 1.19              | 0.58            | 2.05  |            |
| 10   | *Psilocybe subcubensis*        | 8.6 (canopy), 8.0 (stem) | 0.2 (canopy), 0.3 (stem) | 43 (canopy), 26.7 (stem) | [12] |
showed that the contents of psilocin in the canopies and stems of Copelandia were higher than those of psilocybin (0.64–0.74%/0.02–0.22% (psilocin/psilocybin) in the canopies and 0.31–0.78%/0.01–0.39% (psilocin/psilocybin) in the stems), which is consistent with our experimental results. Kamata et al. [16] used LC–MS/MS to analyse the concentration ranges of psilocin and psilocybin in 4 real samples, which were 0.6–1.4 mg/g and 0.18–3.8 mg/g, respectively. The data previously reported in the relevant literature on the contents of psilocin and psilocybin in hallucinogenic mushrooms are summarized in Table 6.

Some drugs are rapidly metabolized in the body, and the parent drug cannot be detected in conventional biological samples such as urine but can be detected in hair [28]. Therefore, hair analysis is a useful tool for retrospective drug screening. The time and length of drug intake can be evaluated by correlating the position of the analyte in the hair sample with the hair growth rate [29].

Conclusions
This paper established a liquid chromatography–tandem mass spectrometry method to detect and quantitatively analyze psilocin and psilocybin in human hair. This method was successfully applied to analyze psilocin in two real hair samples. The concentrations of psilocin detected in the hair samples were 161 and 150 pg/mg, respectively, while psilocybin was not detected. To our knowledge, this is the first demonstration of psilocin in hair specimens of hallucinogenic mushroom consumers. In addition, distribution of psilocin and psilocybin was given in seven mushroom seizure cases. The present method is most sensitive for quantitative analysis of psilocin and psilocybin in human hair among those so far reported.

Acknowledgements This study was supported by grants from Shanghai Science and Technology Commission (19DZ1200600), National Natural Science Foundation of China (81971789, 81772022), Research Institute Projects (GY2019G-4 and GY2020Z-2), Shanghai Key Laboratory of Forensic Medicine (17DZ2273200) and Shanghai Forensic Service Platform (19DZ2292700).

Compliance with ethical standards
Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of authors.

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