Development of an LC-MS/MS method for the identification of psilocybin and psilocin from seized drugs

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Introduction

The growing popularity of illegal edible mushroom food products has created a significant challenge for seized drug laboratories because their complex nature is not suitable for traditional analysis [1]. The complex matrices of edible drugs complicate extraction and subsequent conventional gas chromatography-mass spectrometry (GC-MS) analysis. Specifically, mushrooms that contain psilocybin and psilocin are particularly challenging because psilocybin will thermally degrade in the elevated temperatures of the inlet and oven. Therefore, derivatization is required for samples suspected of containing psilocybin. An LC-MS/MS method was developed for the identification and separation of psilocybin and psilocin. This eliminates the need to derivatize thermally unstable compounds and with a robust technique can accommodate complex matrices like edibles. The method will be tested using the matrices commonly seen with suspected mushroom edibles to ensure signal is not detected.

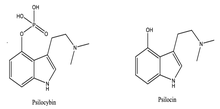

Figure 1: Chemical structures of psilocybin and psilocin

Instrument Parameters

Two multiple reaction monitoring (MRM) transitions were optimized for each analyte as follows: psilocybin m/z 285.4/240.1 and m/z 285.4/58.1, and psilocin m/z 205.2/58.1 and m/z 205.2/160.1. Factors including column temperature, capillary voltage, gradient elution and flow rate were optimized for baseline separation of psilocybin and psilocin. The gradient elution was optimized as seen in **Figure 2**.

Instrumentation

- Agilent 1290 Infinity II Liquid Chromatograph coupled to Sciex Q-Trap ABI 3200 MS and Analyst Software v 1.6.1.

Source Parameters	Value
Gas Temperature (°C)	41
Capillary Voltage	5500
Nebulizing Gas Flow	15 L/min
Heating Gas Flow	35 L/min
Cycle Count	200

Column

- Ascentis® Express 90A biphenyl column (2.7 µm x 50 mm x 2.1 mm) with matching guard column

Mobile Phase

- A: 0.1% formic acid in H_2O
- B: 0.1% formic acid in acetonitrile

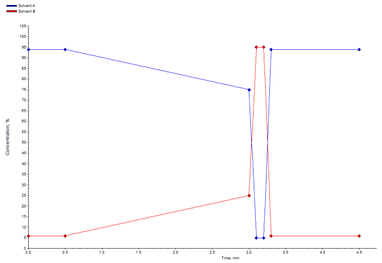

Figure 2: Elution Gradient

The elution gradient as seen in Figure 2 starts at 6% B from 0.0-1.0 mins and is increased to 25% B from 1.0 to 3.0 mins, increased to 95% from 3.0 to 3.1 mins, and held at 95% until 3.2 mins. From 3.2 to 3.3 mins B is decreased to 6% B and held until 4.5 mins when the method runs to completion.

Results and Discussion

Figure 3: Total ion chromatogram of psilocybin and psilocin at 400 and 200 ng/µL, respectively with internal standards psilocybin-D4 (150 ng/µL) and psilocin-D10 (15 ng/µL), showing baseline separation

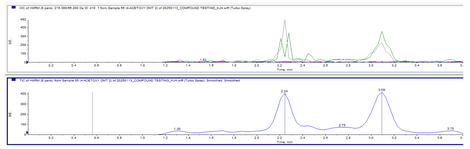

Figure 4: 4-Acetoxy-DMT (100 ng/µL) generated peaks for psilocin transitions 205.2/58.1 and 205.2/160.1 at 2.24 min and 3.09 min. Psilocin can be differentiated from 4-acetoxy-DMT by the presence of the peak at 3.09 min.

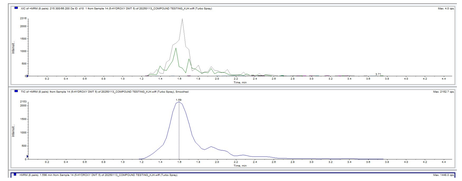

Figure 5: 5-Hydroxy-DMT(100 ng/µL) generated a peak for psilocin transitions 205.2/58.1 and 205.2/160.1 at 1.59 mins

Table 1: Summary of compounds used for interference study

Compound	Detection
N,N-DMT	No peaks present
Delta-9-THC	No peaks present
(6aR,9R)-Delta-10-THC	No peaks present
Cannabidiol	No peaks present
(6aR,9)-Delta-10-THC	No peaks present
4-Acetoxy-DMT	Peaks present at 2.24 and 3.09 mins
5-IT	No peaks present
Delta-8-THC	No peaks present
5-Methoxy-DMT	No peaks present
Norbaeocystin	No peaks present
5-Hydroxy-DMT	Peak present 1.59 mins

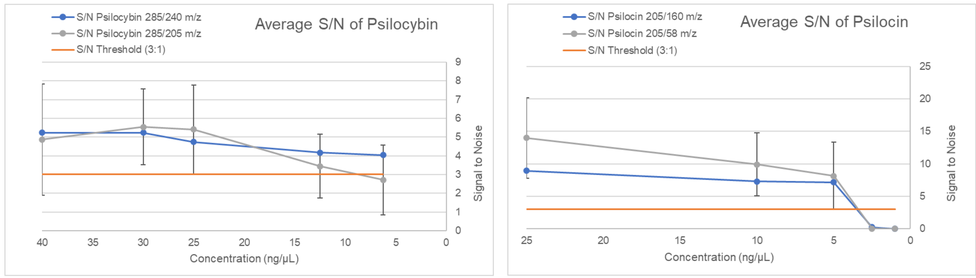

Figure 6: Average signal to noise ratio of psilocybin and psilocin (n=15); error bars indicate 2SD range.

Materials and Methods

All standards used in this study were purchased from Cayman Chemical. Once optimized, the method was evaluated using 4 criteria: selectivity, interference, limit of detection (LOD), and matrix interference.

The selectivity of the method was evaluated by preparing each analyte and their matching internal standard at a concentration of 100 ng/µL for psilocybin and 150 ng/µL for psilocybin-D4, 50 ng/µL for psilocin and 15 ng/µL for psilocin-D10 (Figure 3) run alone and in a mixture.

The interference of this method was tested by using 11 compounds structurally similar to psilocybin or psilocin, or commonly co-detected in casework samples with psilocybin or psilocin. Compounds were prepared at a concentration of 100 ng/µL by dilution with methanol, acetonitrile or 1:1 acetonitrile:H_2O as applicable according to manufacturer recommendations for stable storage.

The LOD was determined by creating 5 standards using serial dilutions for each analyte with the analytical range of 40 ng/µL - 6.25 ng/µL for psilocybin and 25 ng/µL - 1 ng/µL for psilocin. Each standard was analyzed 5 times across 3 consecutive days and monitored for acceptance criteria. Acceptance criteria was defined by maintaining Gaussian peak shape with a signal to noise ratio above 3:1.

Matrix interference evaluation was tested by analyzing 20 samples of blank matrices mimicking edible matrices. Five (5) milk chocolate and five (5) cookies & crème chocolate bars were extracted using an adapted extraction involving acid and base extraction steps as published by the Illinois State Police [4] . Five (5) gummy worm candies and five (5) gummy bear candies were extracted using an adapted procedure from Virginia Department of Forensic Science Controlled Substance Laboratory [5] using acid and base extraction steps. Both adapted extraction procedures have been validated at HCIFS and approved for use. All samples and blanks were run using the developed method and system pressure was monitored for indication of contamination of the column.

Conclusions

- A method for the separation and detection of psilocybin and psilocin was developed for qualitative identification using LC-MS/MS.
- Psilocybin and psilocin can be differentiated from 11 compounds that are either structurally similar or commonly co-detected in casework samples.
- The estimated LODs were 12.5 ng/µL for psilocybin and 5 ng/µL for psilocin
- The chocolate and gummy candies do not show peaks or signal in the analytical range where psilocin and psilocybin elute. The data resembled a blank.
- Future work includes validating the instrumentation and method to be implemented into the HCIFS workflow for edible matrices containing psilocybin and psilocin.

References

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