Quantification of psilocybin and psilocin in mushroom by LC-MS/MS

Karl A. Oetjen[1], Simon C. Roberts[1], Diana Tran[1], Ty Garber[2], Scott Krepich[3], KC Hyland[2], and Christopher D. Borton[1]

[1] SCIEX, USA, [2] Phenova, USA, [3] Phenomenex, USA

Psilocybin and psilocin are psychoactive compounds produced by numerous mushroom species. Historically, these mushrooms were used for spiritual and religious ceremonies in central and southern America, but recently there has been research related to using psilocybin and psilocin to treat addiction and depression. Currently, psilocybin and psilocin are defined as Schedule 1 illicit drugs under the United States Controlled Substances Act.[1,2] However, in Denver, Colorado, the possession of psychotropic mushrooms has been decriminalized. While the selling and purchase of these drugs remain illegal,[3] many see this as a roadmap to eventual legalization, similar to cannabis. Due to the increased interest in these compounds, robust and sensitive analytical methods are needed.

In this study, a 5-minute LC-MS/MS method has been developed for the quantification of psilocybin and psilocin in mushroom matrices.

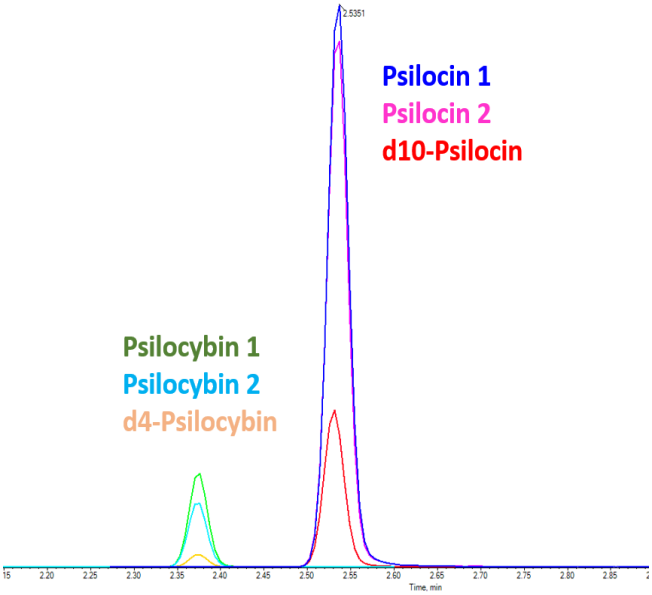

Figure 1. Chromatogram of psilocybin and psilocin in mushroom matrix (M).

Key features of LC-MS/MS System for psilocin and psilocybin analysis

- Limits of detection below 1 ppb in mushroom extract using the SCIEX Triple Quad™ 3500 LC-MS/MS System
- Simple dilute and shoot sample preparation
- Fast analysis time of 5 minutes
- Retention of polar psilocybin and psilocin
- Reproducible quantitative results

Experimental

Sample preparation: Analytical standards for both native and deuterated psilocybin and psilocin were purchased from Cerilliant (TX, USA). Two different mushroom extractions are detailed: the first screens for illicit psilocybin and psilocin in unknown mushroom matrices and the second confirms and quantifies psilocybin and psilocin in actual hallucinogenic mushrooms.

1) Screening and detection of psilocybin and psilocin in unknown mushroom matrices (M): An initial sample weight of 100 mg fresh homogenized shiitake mushroom is extracted in 10 mL of methanol and vortexed. The mushroom matrix was left to further extract at 4ºC overnight. The extract was then spiked with psilocybin and psilocin, with the spiked amount represented as concentration in-vial. Deuterated internal standards were added to each vial.

2) Diluted mushroom (DM) for quantification and confirmation: The diluted mushroom (DM) matrix was diluted to replicate the dilution factor needed to accurately quantify psilocybin and psilocin in real hallucinogenic mushrooms.[1] The 1:100 mushroom extract is further diluted another 1:400 for a total dilution factor of 1:40,000. This large dilution volume is necessary to linearly quantify 3% by sample weight psilocybin and psilocin in hallucinogenic mushrooms.

Chromatography: An injection volume of 2 µL was separated on a Phenomenex Luna Omega Polar C18 (4.6 µm x 150 mm) using mobile phases of formic acid, water, and acetonitrile at a flow rate of 1.2 mL/min.

Table 1. LC gradient.

Time (min)	B (%)
0	*5*
3	*100*
4	*100*
4.1	*5*
5	*End*

Mobile Phase A - 0.1% formic acid in water
Mobile Phase B - 0.1% formic acid in acetonitrile

Mass spectrometry: Both compounds were analyzed in positive polarity on a SCIEX Triple Quad 3500 System using MRM scan mode. Each analyte had a peak width of less than six seconds and at least ten scans for accurate quantification.

Table 2. Source parameters optimized for analysis.

Source parameter	Optimized value
Curtain Gas	*40*
Ion Spray Voltage	*3500*
CAD Gas	*11*
Heater Temperature	*600*
Nebulizer Gas (GS1)	*50*

Discussion

The matrix matched calibration curve exhibited good accuracy within +/- 30% of the expected values for all points, accuracy within +/- 10% for the lowest calibrator, and R^2 coefficients of >0.990. The linear range for psilocybin was from 1 to 250 ppb and for psilocin was 0.1 to 250 ppb in vial (Figure 2). The calibration curve for method one corresponds to 100 ng/mL to 25,000 ng/mL of psilocybin and 10 ng/mL to 25,000 ng/mL of psilocin in unknown mushroom matrices. The calibration curve for method two corresponds to 0.04 to 40 mg/mL of psilocybin (Figure 3) and 0.004 to 10 mg/mL of psilocin (Figure 4) in psychedelic mushrooms. This is an ideal range, as Gambaro *et al.* (2015) showed psychedelic mushrooms have an average of 2.1 mg/mL of psilocin and 1.1 mg/mL of psilocybin.[1] Sensitivity of the SCIEX Triple Quad 3500 System and the robust 5 minute method make this the ideal instrument for this type of analysis.

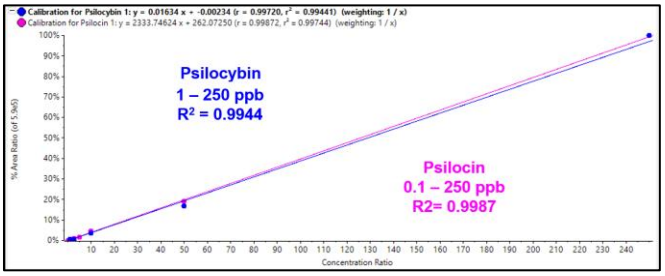

Figure 2. Representative calibration curves of psilocybin and psilocin.

References

1. Gambaro, Veniero *et al.* (2015) Identification of Hallucinogenic Mushrooms Seized on the Illegal Market Using a DNA-Based Approach and LC-MS/MS Determination of Psilocybin and Psilocin. *Journal of Analytical and BioAnalytical Techniques.* ***6*** **(6)**, 578–585.
2. Drug Enforcement Administration (DEA). The Controlled Substances Act. (n.d.). Retrieved from https://www.dea.gov/controlled-substances-act. Nov.2019.
3. Colorado Legal Defense Group. Retrieved from https://www.shouselaw.com/colorado/drugs/CO_psilocybin_mushrooms.html. Nov. 2019.

Psilocybin

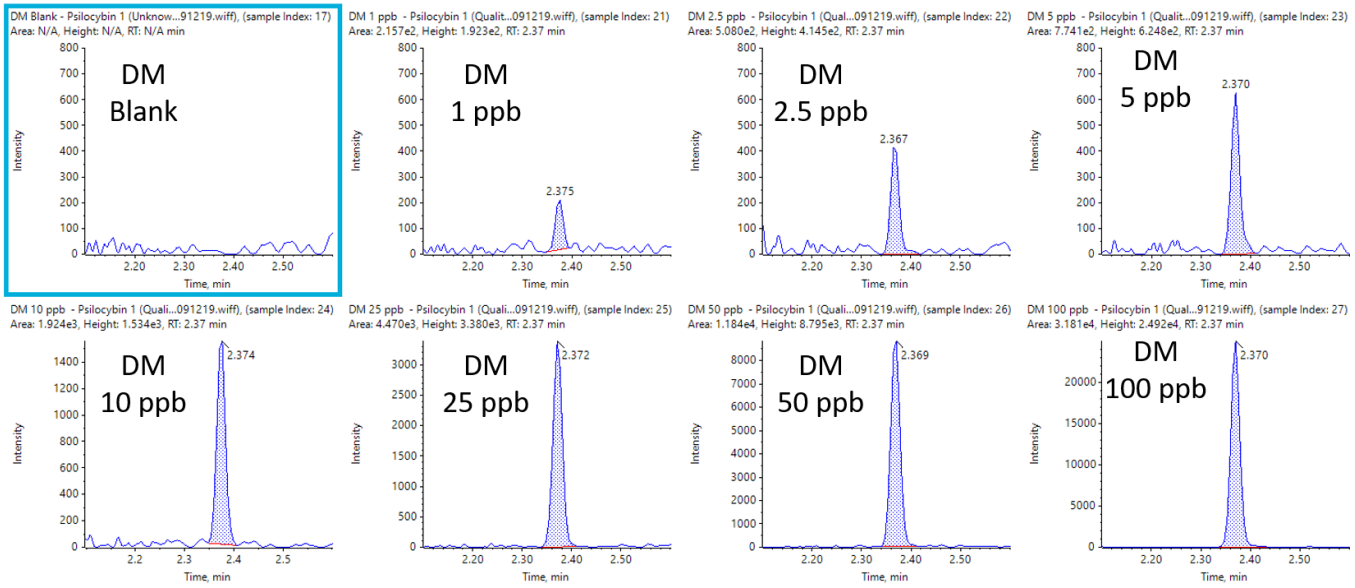

Figure 3. Psilocybin spiked into varying concentrations in diluted mushroom matrix. Limits of quantification were found to be 1 ppb in vial.

Psilocin

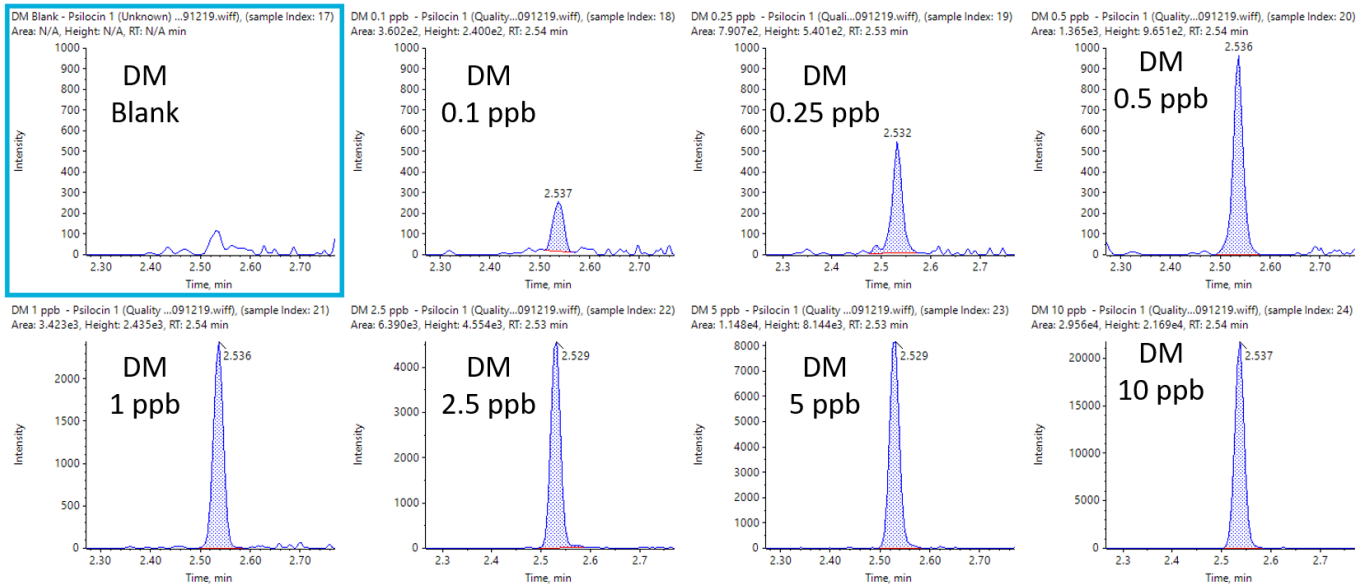

Figure 4. Psilocin spiked into varying concentrations in diluted mushroom matrix. Limits of quantification were found to be 0.1 ppb in vial.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices