

# Identification of Psychotropic Substances in Mushrooms and Chocolate by UHPLC/MS

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## Key Words

- Accela UHPLC System
- MSQ Plus MS Detector
- Magic Mushroom
- Toxicology: Forensics
- Psilocybin: Psilocin

## Goal

Develop a UHPLC/MS method for the detection, separation and confirmation of psilocybin and psilocin in mushrooms and chocolate matrices.

## Introduction

The identification of controlled substances in food matrices is a challenge for forensic laboratories. Classical techniques, such as color tests and thin-layer chromatography, do not provide molecular structural information and cannot be used as a principal means of identification. Infrared spectroscopy (IR) and gas chromatography/mass spectrometry (GC/MS) can identify controlled substances, but suffer from several shortcomings. For example, IR analysis requires that the substances be pure for identification of the controlled substances; clean-up methods are cumbersome and demand excess sample, especially in light of the legal requirement to leave more than half of the exhibit available for subsequent testing. GC/MS methods, the typical screening approach for controlled substances, suffer from coelution of small molecules present in food and beverage matrices. Worse yet, a sample with a high concentration of cholesterol, fatty acids, or caffeine can contaminate the GC/MS instrument, forcing the analyst to clean the instrument and reanalyze all subsequent samples. Some compounds, such as psilocybin, a component of the ‘magic mushroom’ commonly found in food matrices, are thermally labile and do not survive the conditions of GC/MS intact.

To ameliorate these shortcomings, extensive wet-chemistry preparation methods have been developed; unfortunately, they are time-consuming and often require greater amounts of the controlled substances than are present in the evidence. These preparation schemes also do not eliminate some of the more problematic small molecules, and can exclude controlled substances during the course of the separation.

Given the increasing emphasis on instrumental methods of analysis and the limitations of the traditional instruments in the field of forensics, another solution is necessary. LC/MS holds several advantages over the traditional methods of analysis. For example, psilocybin does not decompose at the lower temperatures used in HPLC. The low concentrations of psilocybin and psilocin are not an issue due to increased sensitivity of this technique. Many matrix components that interfere in GC/MS methods do not interfere in HPLC methods because of greater differences in analyte solubility as compared to analyte volatility. However, the employment of traditional HPLC has been limited by lack of resolving power compared to capillary GC. Ultra high performance liquid chromatography (UHPLC) performs separations with improved efficiency and resolution with the use of sub-2  $\mu\text{m}$  diameter particles. The 1-2 second peak widths and high resolving power are more competitive with capillary GC. In this application note, we identify, separate and confirm psilocybin and psilocin in mushroom and chocolate matrices using UHPLC/MS. The psychotropic substances are separated within 5 minutes on a Hypersil GOLD™ PFP 1.9  $\mu\text{m}$ , 100 x 2.1 mm column and detected by a fast scanning single quadrupole mass spectrometer.

## Experimental Conditions

### 1. Sample Preparation

Psilocybin (Lot #284) and psilocin (Lot #383) standards were purchased from Alltech (State College, PA, USA) and used as received. These standard compounds were mixed and diluted to about 50 ppb with methanol as stock standard solution. The psilocybin mushrooms were taken from a training sample and the chocolates were from returned evidence after adjudication.

Two (2) mL methanol was added to 10 mg dried magic mushroom. This mixture was vortexed for 30 seconds before the supernatant was filtered through a cotton-plugged Pasteur pipette, centrifuged for 90 seconds, and filtered again. The supernatant was further diluted 50-fold with methanol prior to analysis.

The chocolate samples were prepared using 10 mg of chocolate material, and the same extraction as for the mushroom material.

## 2. Chromatographic Conditions

Chromatographic analyzes were performed using the Accela™ UHPLC system (Thermo Scientific, San Jose, CA). The chromatographic conditions were as follows:

Column:	Hypersil GOLD PFP (perfluorinated phenyl) 1.9 μm, 100 x 2.1 mm			
Flow Rate:	1 mL/min			
Mobile Phase:	A: Water with 0.06 % acetic acid B: Acetonitrile with 0.06% acetic acid C: Methanol with 0.06% acetic acid			
Gradient:	T (min)	A%	B%	C%
	0	95	0	5
	3.0	5	5	90
	4.0	5	5	90
	4.1	95	0	5
	5.0	95	0	5
Injection Volume:	2 μL partial loop injection, 25 μL loop size			
Column Temp:	45 °C			

## 3. Mass Spectrometer Conditions

MS analysis was carried out on a MSQ Plus single quadrupole LC/MS detector (Thermo Scientific, San Jose, CA). The MS conditions were as follows:

Ionization:	Electrospray (ESI)
Polarity:	Positive
Probe Temp:	500 °C
Cone Voltage:	90 V
Scan Mode:	Full scan with mass range of 100-400 <i>m/z</i>
ESI Voltage:	3.5 kV
Scan Time:	0.2 s

## Results

Psilocybin and psilocin standards elute rapidly at 0.65 min and 2.25 min respectively with excellent resolution (Figure 1). Detection was carried out using full scans (100-400 *m/z*) of the single quadrupole mass spectrometer. Molecular ions of each compound (*m/z* 285 and *m/z* 205 for psilocybin and psilocin respectively) were observed. Authentic mushroom samples, taken from a training sample known to contain psilocybin and psilocin, were prepared by a series of extractions and filtrations as described above. These mushroom samples were analyzed using the same method developed for the standards and showed peaks with retention times consistent with the psilocybin and psilocin standards (Figure 2). The identities of peaks at 0.64 min and 2.22 min in figure 2 were also confirmed as psilocybin and psilocin by MS detection. The chocolate samples, which were taken from an adjudicated case and were known to contain psilocybin and/or psilocin, also showed the presence of both psilocybin and psilocin (Figure 3). Blanks were run successively after each run, with no apparent carryover from one run to the next.

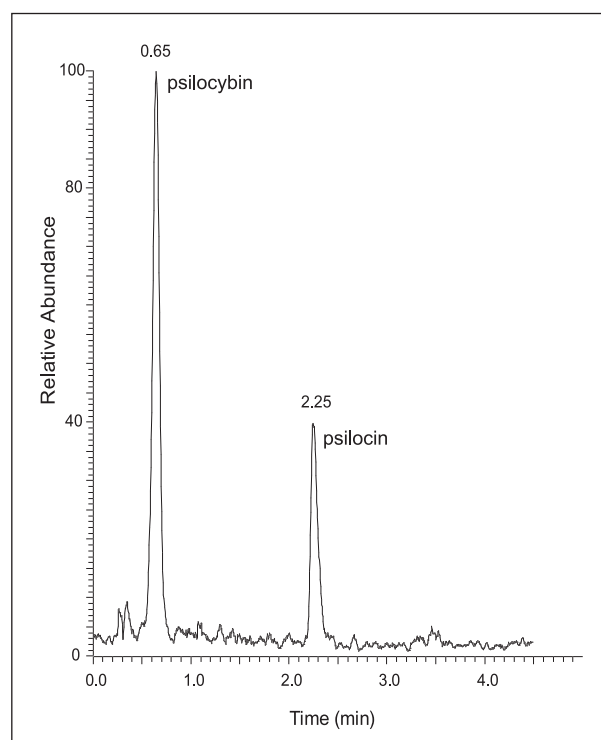


Figure 1: Psilocybin and psilocin standards

## Conclusion

UHPLC/MS is an excellent means of identifying psilocybin and psilocin in both raw mushrooms and chocolates. The preparation time (10 minutes) and run time (10 minutes including blank) make this a very efficient analytical method.

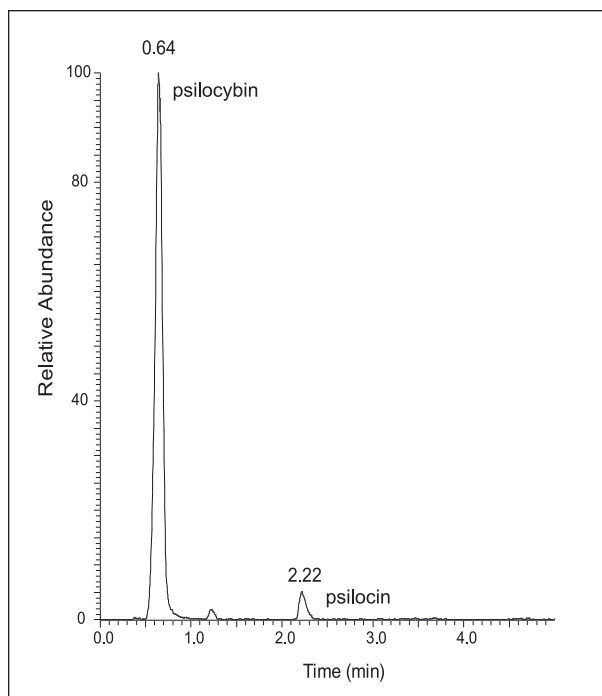


Figure 2: Methanol extract of raw mushroom material

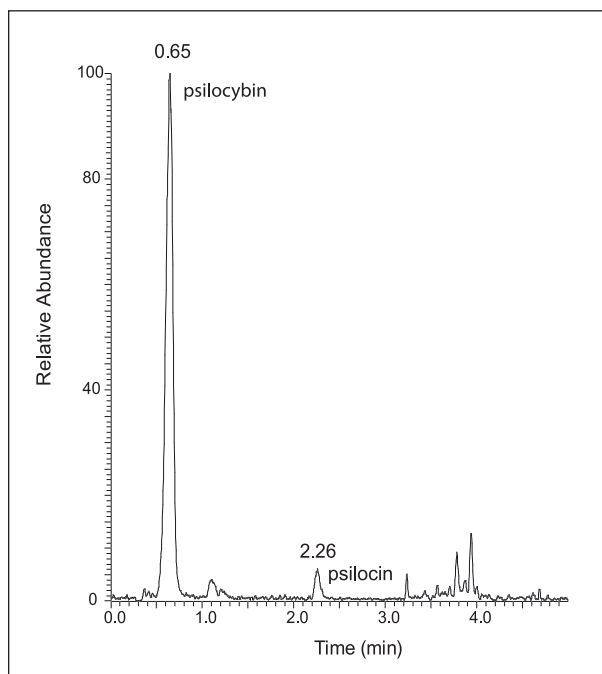


Figure 3: Methanol extract of chocolates containing psilocybin and psilocin

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