



# TENNESSEE BUREAU OF INVESTIGATION

## Forensic Services Division

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### Forensic Chemistry Standard Operating Procedure Manual

#### Psilocyn and Psilocybin

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## 29.0 PSILOCYN AND PSILOCYBIN

### 29.1 Background

Psilocyn and psilocybin are the controlled hallucinogenic components in many species of mushrooms native to the United States, Mexico and South America.

The analysis of hallucinogenic mushroom material can be a challenge. Psilocyn, psilocybin, or both may be present in hallucinogenic mushroom samples. However, psilocybin is more stable and may be more predominate in dried mushroom material. Psilocybin will convert to psilocyn by losing a phosphate group when exposed to high heat, a strong acid, or a strong base. Other extraction schemes may cause this conversion as well.

### 29.2 Testing Procedures

Reagents can vary for individual analytical techniques. See the methods below for specific reagents used in extraction.

The TLC solvent system mixture consists of 50 ml of N-propanol, 25 ml of acetic acid, and 25 ml of high purity water for a total of 100 ml (2:1:1).

Refer to Appendix A for preparation instructions for PDMAB spray.

#### 29.2.1 Extractions

Approximately 250 mg of dried mushroom material is ground, cut, or torn into small pieces and mixed with an approximately equal amount of methanol or reagent alcohol. The sample is then vortexed for about one minute. If the sample is not dry, it should be placed in an oven at approximately 80°C for about thirty minutes. It may also be necessary to concentrate the extracted sample by blowing a stream of cool air or nitrogen over it. Extractions can be dried and dissolved in methanol or other solvent as needed.

If there are compounds in the sample that interfere with identification, a basic extract of the sample into 1,2-dichloroethane or diethyl ether may be utilized. This extraction will result in the conversion of any psilocybin to psilocyn. This extract cannot be used for the TLC or HPLC analysis. Micro-filtration of the sample can also aid in the removal of interfering compounds.

#### 29.2.2 Presumptive testing

Screening can be conducted using the UV spectrophotometer on the extract of the sample. This test is performed by taking an aliquot of the extraction (two or three drops) and placing it in a cuvette of methanol.

A TLC examination of the methanol extract may be performed by spotting it on pre-coated silica gel thin layer plates along with psilocybin and psilocyn standards. Develop the plates in the mushroom solvent system. Dry the plates with a stream of warm air from a hair dryer, and locate the spots for the sample and standards by spraying with PDMAB spray. If the sample is



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psilocybin mushrooms, one or two spots will develop matching the psilocybin or psilocyn standards or both in color and distance traveled on the plates.

#### **29.2.3 Confirmatory Testing**

LC/MS or TLC in conjunction with a GC/MS will be considered confirmatory tests for psilocybin if present in the sample.

An analyst may use GC-IR, GC retention times, UV/Vis, Marquis color tests, or PDMAB color tests in conjunction with a GC-MS and report "Psilocybin and/or Psilocyn" in the results since no determination can be due to potential psilocybin degradation from these testing methods.

#### **29.3 Special considerations**

Mushroom exhibits that have a net weight less than 1.00 grams are considered insufficient for analysis since 0.25 grams of the substance are required for each sampling due to the low concentrations that are present within most psilocybe mushrooms. Mushroom exhibits that are under this weight requirement will not satisfy the requirements outlined in section 10.4.3.

Improper packaging or handling of mushroom material may decompose the sample to the extent that psilocyn and psilocybin can no longer be detected.

Mushrooms are typically encountered in a dried form. Mushroom material may be incorporated into other materials, such as chocolate brownies. A visual examination of the substance may be necessary to separate the mushroom material prior to testing.

#### **29.4 References**

Beug, Michael W. and Bigwood, Jeremy, *Quantitative Analysis of Psilocybin and Psilocyn in Psilocybe Baeocystis (Singer and Smith) by High-Performance Liquid Chromatography and by thin-Layer Chromatography*, Journal of Chromatography, 207, 1981, pp. 379-385.

Carman, D. and Drowns, K., *Isolation and Identification of Psilocybin and Psilocyn from Mushrooms*, SAFS Meeting, Chattanooga, TN, 1983.

Oregon State Police, *Controlled Substances Procedure Manual*, Revision, 32. Jan 11, 2011.