

#8

Psilocyn/Psilocybin Mushrooms

Analytical Methods

1.0.0 Background

Psilocyn and psilocybin are related tryptamines that are found in many species of mushrooms. The mushrooms have been used in religious ceremonies for at least 3000 years by the native peoples of Mexico and Central America. Psilocyn and psilocybin are Schedule I hallucinogens. More information is available through the "Drug Identification Bible".

2.0.0 Scope

The following procedures are used to identify psilocyn and or psilocybin from mushrooms.

3.0.0 Equipment and Reagents

The following equipment can be used to identify the analytes of interest.

- 3.1.0 A GC/MS and appropriate analytical software. Reference GC/MS AM.
- 3.2.0 ACS grade solvents, methanol and acetone.
- 3.3.0 Fast Blue BB, or B salt
- 3.4.0 Deionized/ distilled water
- 3.5.0 Hydrochloric acid

4.0.0 Color Spot Test

4.1.0 "Weber test" "The Weber Test for the Presence of Psilocyn in Mushrooms", Garrette, Siemens, and Gaskill, *NEAFS* vol. XVIII, No.1, 1993.

- 4.1.1 Add sample to well of spot plate after the addition of a Fast blue BB, or B, solution. Should turn orange-red within a couple of minutes if psilocin/psilocybin is present.
- 4.1.2 Remove some of the liquid to another well and then add a drop of concentrated HCl. A positive test is one that turns a blue-green color.
- 4.1.3 Negative and positive controls need to be run with each batch, and the results documented in the case notes.

5.0.0 GC/MS Sample Preparation and Analysis

- 5.1.0 Extraction.
 - 5.1.1 Extract with just enough methanol to cover sample. **NOTE** At this stage the methanolic extract may be injected into the GC/MS.
 - 5.1.2 Centrifuge and decant solution into clean test tube. Cap and place into freezer for at least one hour.
 - 5.1.3 Remove from freezer and immediately add equal volume of acetone and mix.
 - 5.1.4 Centrifuge, decant, and if necessary concentrate the supernatant.

- 5.2.0 Analysis.
 - 5.2.1 Run samples on GC/MS using a split or splitless data acquisition method depending on the sensitivity of the instrument.
 - 5.2.2 Compare with a standard of either psilocyn or psilocybin. NOTE psilocybin breaks down into psilocyn in the hot injection port of a GC.
- 5.3.0 Conclusions and Reporting.
 - 5.3.1 Confirmation. The retention time must be within 0.04 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied then confirmation can be reported as “psilocyn and/or psilocybin (CI)”.

4.0.0 Thin Layer Chromatography

If differentiation of psilocyn and psilocybin is required then a T1 system (10mls methanol + 7 drops of NH₄OH), developed with PDMAB, works well.

5.0.0 History

Revision #	Issue or review date	History	Author or Reviewer
0	7/22/02	Original Issue	D.C. Sincerbeaux
1	8/27/02	Scope & #	D.C. Sincerbeaux
2	9/13/05	4.1.2 and 4.1.4	D.C. Sincerbeaux
3	1/12/07	Added history & page #s dropped grinding requirement, 4.1.1 & 5.1.1	D.C. Sincerbeaux
4	7/2/12	changed 5.1.1	D.C. Sincerbeaux
5	9/16/14	Slight change to 5.3.1	D.C. Sincerbeaux