Introduction

Several species of mushrooms contain the hallucinogens psilocyn and psilocybin. Under the Florida Statutes, these hallucinogens are classified in Section I of Chapter 893, Controlled Substances. Two major factors complicate the identification of these compounds in mushrooms. First, chromatographic problems arise due to large amounts of alkaloids and sugars present. Second, dephosphorylation of psilocybin to psilocyn occurs due to the hydrolytic instability of psilocybin in addition to the thermal and catalytic lability of this drug by gas chromatography/mass spectrometry (GC/MS). Procedures are geared toward the analysis of the decomposition product of psilocybin, which is psilocyn.

Safety

- Personal protective equipment (PPE) including safety glasses/safety shield, gloves, and lab coat/scrubs should be worn when working with the potentially hazardous materials in this procedure. A fume hood must always be used when working with hazardous gases, solvents, dust, etc. Additional protective equipment may be used at the discretion of the analyst. Appropriate personal protective equipment is specified in the Safety Section of the Quality Assurance Manual (QAM).

- Disposal of all chemical and biohazardous waste must be performed in accordance with the procedures detailed in the Safety Section of the QAM.

Extraction Procedures

Three separate validated extraction procedures are available for use. The chemist has discretion to use either Extraction Procedure 1, 2 or 3.

Extraction Procedure 1

- If mushrooms are not dried, place the mushrooms in an oven at 40 ºC for approximately 2 – 4 hours or leave in the hood until dry (crispy).

- Clean and rinse blender with methanol and discard rinse. Using clean blender jar, add 150 mL of methanol and mix for 3 minutes. Filter methanol solution using a Buchner funnel with vacuum and #1 filter paper. Transfer methanol to a beaker and set aside. This will be the extraction blank.

- Take approximately 2.0 – 6.0 g of dried mushrooms and pulverize with a blender to a fine powder.

- Add 150 mL of methanol to the powdered mushrooms in a blender and mix for 3 minutes. Allow the suspended material to settle.

- Filter the extraction blank followed by the sample extract using a Buchner funnel with vacuum and #1 filter paper. Transfer each methanol solution to a clean beaker.

- Evaporate both beakers. The sample beaker should be evaporated almost to dryness. Heat gun and hot plate (setting #3) may be used. Mushroom extract may be an oily liquid.

- For each beaker: Add 10 mL of 0.1 N H_2SO_4 to the beaker and agitate. The entire residue of the sample beaker will not dissolve. Decant the H_2SO_4 to a clean 15 mL extraction tube. Rinse a second time with 5.0 mL of the H_2SO_4 and decant the solution.

- Centrifuge and decant the H_2SO_4 solution to a clean 125 mL separatory funnel.

- Wash H_2SO_4 three times with 20 mL of chloroform and discard bottom layer (chloroform layer). Make H_2SO_4 basic with approximately 1-5 mL of concentrated NH_4OH (pH approximately 9). Additional NH_4OH may be necessary.

- Extract three times with 20 mL chloroform, filter through 1 PS paper using a small amount of powdered Na_2SO_4 and evaporate to dryness. Allow last of chloroform to evaporate. Heat gun and hot plate (setting #3) may be used.

Extraction Procedure 2

- Place mushrooms in an oven at 40 ºC for approximately 2-4 hours or leave in the hood until dry.

- Add 10% acetic acid solution to a blender. Mix for approximately 1 minute and transfer to a beaker then to centrifuge tube(s). This will be the extraction blank.

- Take approximately 1.0 g of dried mushrooms and pulverize with a dry blender to a fine powder. Add enough of a 10% acetic acid solution to just cover the mushrooms.

- Mix for approximately 30 seconds, add 5 mL of deionized water and mix an additional 30 seconds. Mixture should be fine slurry.

- Transfer the slurry to a beaker and then to centrifuge tube(s).

- Centrifuge extracts for 3 minutes.

- Transfer the supernatant into large beakers.

- Neutralize the supernatant by adding small amounts of sodium bicarbonate. This may take several grams of sodium bicarbonate.

- Test the pH to ensure neutralization is complete (or when foamy effervescence stops).

- Transfer the resulting solution into a centrifuge tube and extract with equal amounts of chloroform.

- Centrifuge for 3 minutes.
- Remove chloroform layer and collect in a scintillation vial.
- Concentrate the chloroform extract. Heat gun and hot plate (setting #3) may be used.
- Add a few drops to perform the spot test on filter paper. Once spot test is performed, add internal standard and transfer the rest to a micro vial for GC and GC/MS analysis.

Extraction Procedure 3

- If mushrooms are not dry, then place in oven for 2-4 hours or leave in hood until dry.
- If sample size permits, homogenize a portion of the material and then sample 1 gram from the homogenized sample.
- Using a mortar, add 1 g of sodium bicarbonate and just enough distilled water to make a paste (use a squirt bottle and do not use an excess amount of water). Add 10 mL of ethyl ether to mortar to rinse. Use a glass pipet to remove the ethyl ether layer and transfer to a scintillation vial. Repeat rinse twice with 5mL ethyl ether. Transfer each ethyl ether rinse to the same scintillation vial and evaporate to dryness using heat. This will serve as the extraction blank.
- Clean the mortar used for the extraction blank and use the same mortar for the mushroom extraction.
- Grind 1 g of mushrooms with the mortar and pestle.
- Add 1 g of sodium bicarbonate.
- Using a squirt bottle add a small amount of distilled water, enough to make a paste. Do not add an excess amount of water.
- Add 10 mL of ethyl ether and stir. Use a glass pipet to remove the ethyl ether layer and transfer to a scintillation vial.
- Repeat twice with 5mL ethyl ether. Transfer each ethyl ether rinse to the same scintillation vial.
- Evaporate extract in scintillation vial to dryness using heat.

- Reconstitute with ~250 uL of internal standard and transfer to an ALS vial containing a microvial insert.

- Allow extracted mushroom waste to dry in hood and then discard.

Spot Color Testing

- Add a small amount of extracted sample to filter paper and dry.
- Place a few drops of the Van Urks Reagent (PDAB) and place on minimal heat. A purple color should appear from where the sample was spotted indicating a positive reaction for an indole type compound. Psilocyn can give a blue-grey color and psilocybin can give a reddish-brown color. If no color develops then either the sample size was too small or they are not psilocybin-containing mushrooms.

Instrumental Analysis

- **Gas Chromatography/Mass Spectrometry Analysis:**
  - Analyze the sample and standard along with the appropriate blanks using GC/MS with the QSCREN method.
  - Compare the spectra of the sample with the standard by looking at the overall pattern for a positive confirmation.

References

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