Introduction

The Florida state defined limit specifies that for a *Cannabis sativa* plant to be considered hemp, the plant does not have a delta-9-tetrahydrocannabinol (THC) concentration exceeding 0.3 percent on a dry weight basis. This method is designed to determine if a plant contains at least 1% THC (which is more than three times the state defined threshold). No effort is made to ensure 100% extraction efficiency or to determine the actual percentage of total THC present in the plant.

Plant material will be analyzed using a macroscopic/microscopic examination and tetrahydrocannabinol (THC) will be qualitatively and semi-quantitatively identified by gas chromatography-mass spectrometry (GC-MS). Optionally, a rapid modified Duquenois-Levine color test may be performed. The total amount of THC in the plant is defined as the combined THC and tetrahydrocannabinolic acid (THCA) concentration. Since THCA is converted to THC in the GC injection port, the method does not differentiate between THC and THCA. Using a 50 mg plant sample, 1% THC equates to 0.5 mg of THC extracted from the sample. Samples are extracted in 5 mL of solvent (internal standard solution), resulting in a 0.1 mg/mL solution. The ratio of the THC to internal standard (IS) from the extract is then compared to a known 0.1 mg/mL THC with IS reference standard to determine if the plant sample is over the decision point (over 1% THC).

Plant samples will be screened for CBD:THC ratio by GC-MS to eliminate probable hemp samples prior to proceeding to 1% threshold testing.

Safety

- Personal protective equipment (PPE) including safety glasses/safety shield, gloves, and lab coat/scrubs must 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.

Paraphernalia

- Paraphernalia such as pipes will be analyzed on a case by case basis with approval of the Chemistry Manager. Pipes will be analyzed for marijuana if sufficient plant material is present to test for the presence of Cannabis by performing a macroscopic, microscopic, color test (optional) and threshold testing by GCMS analysis.
• Vaporizers and cartridges containing an oily substance and other materials potentially containing THC will be analyzed by performing the Rapid Modified D-L test and GC/MS if the offense date is prior to 7/1/19.

Macroscopic Examination (Plant Material)

• Gross morphological characteristics that may be observed include the palmate arrangement of the leaflets, the pinnate appearance of the leaflets, the serrated edges of the leaflet, the buds (with or without seeds) and if present, fluted stems and/or stalks.

• Positive macroscopic examination results are recorded in the analytical notes by using the abbreviation 'pos', '+', or 'Y'. Negative macroscopic examination results are recorded in the same location using 'neg', '-', or 'N'. The chemist will also document what macroscopic characteristics were observed leading to the positive result.

• In some cases, the condition of the plant material or quantity of plant material is insufficient to perform macroscopic identification. If there is insufficient sample for macroscopic analysis, then microscopic, color test and GCMS analysis may be performed to confirm Cannabis.

Microscopic Examination (Plant Material):

• Place leafy material under the stereo zoom microscope, and observe the following botanical characteristics:
  
  ➢ Cystolith hairs (bear claw appearance), must be present
  
  ➢ Elongated hairs (underside of leaf), must be present
  
  ➢ Resin glands (glandular hair), may be present

• For the microscopic test to be considered positive, both cystolith and elongated hairs must be present. Positive identification of each type of hair is documented in the analytical notes as 'pos', '+', or 'Y' in the appropriate field. Negative identification of each type of hair is documented in the analytical notes as 'neg', '-', or 'N' in the appropriate field.

Microscopic Examination (Differentiation between Compressed Marijuana and Hashish)

• This examination only needs to be performed when the appearance of the item is similar to compressed marijuana. If item can be visually determined not to be compressed marijuana, this examination does not need to be performed.

• Place a portion of the suspected hashish on a microscopic slide.
• Apply chloroform dropwise and observe under a polarizing microscope.

• Look for an abundance of unattached cystolithic hairs or "bear claws".

• Using the crossed nichols position (polarizer and analyzer 90° to one another), observe the birefringence of the hairs or “bear claws”. The hairs should be bright white in appearance and be easily extinguishable from surrounding leaf material.

• The combination of the loose hairs and the birefringence is highly indicative of hashish and not compressed plant material.

Performing the Rapid Modified Duquenois-Levine Test

• Preparation of the D-L reagent (500 mL): Dissolve 5 mL of acetaldehyde and 10 grams of vanillin in ~95% ethanol to 500 mL in a volumetric flask.

• Place a small portion of the material in a test tube.

• Add approximately 1 mL of the D-L reagent to the material. Optional: After waiting for a few minutes, decant solution off of leaf material into another test tube.

• Add at least equal portions of concentrated hydrochloric acid, as there is D-L reagent to the test tube.

• Observe the color development to purple within a few minutes. If the color does not develop, it is possible that the sample does not contain cannabinoids or the sample may be a seedling, old or moldy. Try extracting the sample with petroleum ether first, drying the ether, then performing the color test.

• Extract the purple color in the aqueous layer with chloroform (CHCl₃) and observe the purple color in the CHCl₃ layer of the test tube.

• The purple colors in both layers have to be observed for the test to be considered positive. Positive results are indicated in the analytical notes as either 'pos', '+', or 'Y'. Negative results are indicated in the analytical notes as either 'neg', '-', or 'N'.

• Results of the macroscopic, microscopic and D-L tests will be documented on the CH Marijuana Form.

CBD:THC Area Ratio Screen

• Samples will initially be screened for CBD:THC area ratio.

• Evaluate the CBD:THC ratio.
CH Cannabis Methodology

- If the CBD peak area is less than the THC peak area, then document that the area was evaluated on the CH Marijuana Form. No calculations are required.
- If the CBD peak area is greater than the THC area:
  - Print the Area Percent Report to be included in the case file.
  - Calculate the CBD:THC ratio on the CH Marijuana Form.
  - A CBD control in testosterone internal standard must be analyzed and included in the case record.

- If the CBD:THC ratio is greater than 20:1 then THC:IS threshold testing calculations are not required.

- If the CBD:THC ratio is less than 20:1 then perform 1% threshold testing calculations.

1% Threshold Testing by Gas Chromatography / Mass Spectrometry (For Cannabis Potentially Containing Tetrahydrocannabinol)

THC Standard Preparation

1. A new standard is prepared each day when an analysis is performed.
2. Add 900 µL of methanol containing the internal standard (testosterone, 0.075 mg/mL) (I/S) using a 100-1000 µL calibrated pipette to an autosampler vial.
3. Using a calibrated 10-100 µL pipette, add 100 µL of certified THC reference material stored in the freezer.
4. Record the information in CIMS including serial numbers of the pipettes used.

Sample Preparation

1. Two samples of plant material of approximately 50 mg (±1 mg) are crumbled with gloved hands and then accurately weighed into two labeled test tubes (seeds, stems, stalks and roots are not included). If the plant material cannot be crumbled, scissors can be used to cut the plant material into fine pieces.
2. The weights are recorded on the CH Weight Form.
3. Each sample is dissolved in 5 mL of methanol containing the internal standard (testosterone, 0.075 mg/mL), inverted once and sonicated for 10 minutes.
4. An extraction blank for each extraction is also performed.
5. The test tubes are then inverted to mix the solution and approximately 1 mL of the IS solution is decanted into an autosampler vial for GC-MS analysis.
THC:IS Area Ratio Calculation

1. Print the Area Percent Report to be included in the case file.
   In the Chemstation menu select Chromatogram -> Percent Report with Chromatogram.

2. Using the formula below, compare the THC:IS area ratios of the case sample to the
   THC:IS area ratio of the standard.

   \[
   \text{THC:IS Area Sample Response Ratio} = \frac{\text{Sample THC (area)}/\text{IS (area)}}{\times [50 \text{ mg}/\text{Amount sampled}]}
   \]

3. Calculate the %CV for the THC:IS area ratios between the duplicate samples analyzed.
   The %CV must be lower than 20% in order to pass. Otherwise, the extraction must be
   repeated.

   \[
   \%CV = \left(\frac{\text{Standard Deviation}}{\text{Mean}}\right) \times 100
   \]

Instrumental Parameters
The 1% THC threshold testing will be performed using a gas chromatograph (GC) coupled
with a mass spectrometer (MS) validated for the THCSREN method.

<table>
<thead>
<tr>
<th>Instrument Name</th>
<th>GC Type</th>
<th>MS Type</th>
<th>Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS 1 / GC-MS 2</td>
<td>Agilent 7890 series</td>
<td>Agilent 5977 series</td>
<td>Agilent HP-5MS UI Part #19091S-433UI; 30m x 0.250 mm i.d. x 0.25 µm film thickness [(5%-phenyl)-methylpolysiloxane]</td>
</tr>
</tbody>
</table>

The instrument method will be maintained in the Chemistry Unit.

Reporting Results
- Plant material: Report will state in similar form or other slight variation as
  - If the CBD:THC area ratio is greater than 20:1 report as “Indicative of Hemp Net Weight: # grams”.
  - If the THC:IS area ratio is less than the THC standard report as “Inconclusive Marijuana/Hemp due to the low concentration of THC Net Weight: # grams”.
  - If the THC:IS area ratio is greater than the THC standard report as: “Marijuana Schedule I Net Weight: # grams”.

- Other THC containing products with an offense date prior to July 1, 2019: Report will state in similar form or other slight variation as TETRAHYDROCANNABINOL Schedule I Net Weight: # grams.”

References

- *Analysis of Drugs Manual*; DEA, U.S. Department of Justice, Office of Forensic Sciences, 2nd ed.
- *Basic Training program for Forensic Drug Chemists*; DEA, U.S. Department of Justice, Office of Forensic Sciences, 3rd ed.
- DEA Method for Qualitative Analysis of Cannabinoids by Gas Chromatography. Drug Enforcement Administration Southeast Laboratory.
• DEA SOP-CH-001. Standard Operating Procedure for the Analysis of Suspected Cannabis Plant Material.
