Chapter 2: Technical Procedure for the Identification of Cannabis Plant Material and Extracts

1. Purpose/Scope

This procedure provides direction for the identification of Cannabis as defined in NC General Statute §90-87 (16) in the Drug Chemistry Unit of the Wake County Bureau of Forensic Services.

2. Definitions

2.1. Reference material - Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

3. Equipment, Materials and Reagents

3.1. Equipment

3.1.1. Leica S9i stereomicroscope(s) equipped with 10X eyepiece and 9:1 zoom capability to produce magnification of 6.1-55X with digital camera and Leica Application Suite (LAS) software.
3.1.2. Nikon Eclipse E400 Pol polarizing microscope equipped with 10X eyepiece and 10X objective to produce magnification of 100X
3.1.3. Gas Chromatograph/Mass Spectrometer (GC-MS)
3.1.4. Fisher Dry Bath Incubator

3.2. Materials and Reagents

3.2.1. Cannabis or Delta-9-Tetrahydrocannabinol reference material
3.2.2. Chloroform, ACS grade
3.2.3. Vanillin, NF
3.2.4. Acetaldehyde, 99.5%
3.2.5. Ethanol, ACS
3.2.6. Culture tube or spot plate
3.2.7. Bis(trimethylsilyl)trifluoroacetamide with 1% Trimethylchlorosilane (BSTFA w/ 1% TMCS)

4. Procedure

4.1. For exhibits with a gross weight of less than 5 grams consisting of hand-rolled cigarettes or partial hand-rolled cigarettes, the paper may be included in the weight recorded and reported. The evidence may be cut open to expose the plant material for viewing and analysis. Use the following statement to report the weight when the paper is included:

"Weight of paper and plant material:"

4.2. Observe plant material macroscopically and microscopically to verify the presence of visually recognizable morphological characteristics. Macroscopic and microscopic morphological characteristics shall be consistent with cannabis reference material characteristics.

4.3. Record the lot number or Drug Chemistry designation of the cannabis reference material used for comparison in the case file.

4.4. Macroscopic characteristics – Record the observed macroscopic characteristics present in the exhibit in the case file. Include any additional details as needed.

4.4.1. An exhibit must contain sufficient macroscopic characteristics described and referenced in this procedure to be macroscopically consistent with cannabis or be visually consistent with cannabis reference material for the macroscopic examination to be considered as a positive Category B test, refer to the Technical Procedures for Drug Chemistry Analysis.

4.4.2. Macroscopic Characteristics

4.4.2.1. Stalks and stems are longitudinally grooved.

4.4.2.2. The plant branches at the nodes – a branch appearing immediately above each leaf. The branches occur at opposite points on the stalk with alternate pairs situated at approximately right angles except at the top of the plant, where the arrangement becomes alternate rather than opposite.

4.4.2.3. Plant has compound palmate leaves with 5-11 leaflets (usually seven), and odd in number.

4.4.2.4. Leaflets are pointed at both ends and vary up to about 6 inches length and to about 1.5 inches in width. They are characteristically hair covered, veined and serrated (with notched edges.)

4.4.2.4.1. The side veins run out from the center vein of the leaf slantwise to the tips of the serrated edges.

4.4.2.4.2. The serrated edges point towards the tips of the leaflets.

4.4.2.4.3. The upper surface is darker than the lower surface.

4.4.2.5. Seeds are about 2 – 5 mm long, greenish-yellow to brown, mottled, covered with lacy markings, ovoid in shape and divided into two segments by a ridge extending around the greatest circumference.

4.4.2.6. Seeds are enclosed in hulls or pods which are green, hairy and sticky to the touch.

4.5. Microscopic Characteristics

4.5.1. Observe the microscopic characteristics using the stereomicroscope and record the observations in the case file. An exhibit must contain leaves that meet the requirements of 4.5.1.3.3 and 4.5.1.3.4. for the microscopic examination to be considered as a positive Category B test, refer to the Technical Procedure for Drug Chemistry Analysis. Digital images of these leaf characteristics shall be taken. Additional images may be taken of additional plant characteristics.

4.5.1.1. The plant has glandular (related to a cell or group of cells that produces a secretion) trichomes (hair-like projections) where the cannabis resin is produced and stored. They are mainly associated with the flower structures but they can also be found on the lower surface of the leaves and occasionally on the stems of young plants.

4.5.1.2. The plant has non-glandular trichomes which are unicellular, rigid and curved with a slender pointed apex.

4.5.1.3. Required characteristics for identification of cannabis leaves:

4.5.1.3.1. Green, brown or brown-spotted in color

4.5.1.3.2. Characteristically veined and serrated

4.5.1.3.3. Non-glandular cystolithic hairs on the upper side with a characteristic bear claw shape with cystoliths, calcium carbonate crystals, visible at their bases.

4.5.1.3.4. Non glandular, non-cystolithic hairs on the lower surface which are longer, more slender and more sharply pointed than the hairs on the upper surface.

4.5.1.4. Required characteristics for identification of cannabis stems:

4.5.1.4.1. Green, brown or brown-spotted in color

4.5.1.4.2. Longitudinally grooved.

4.5.1.5. Required characteristics for identification of cannabis seeds**:**

4.5.1.5.1. Greenish-yellow to brown, mottled

4.5.1.5.2. Covered with lacy markings

4.5.1.5.3. Ovoid in shape

4.5.1.5.4. Ridge around the greatest circumference

4.5.1.6. Required characteristics for identification of cannabis hulls:

4.5.1.6.1. Green, brown or brown-spotted in color

4.5.1.6.2. Characteristically shaped, ovoid

4.5.1.6.3. Non-glandular cystolithic hairs on outer surface

4.5.1.6.4. Glandular hairs which are shaped like clubs with flattened spherical heads

4.6. Color Test

4.6.1. Duquenois-Levine (Modified)

4.6.1.1. Reacts with cannabis/cannabinoids to produce a violet blue color that transfers to the chloroform layer.

4.6.1.2. Preparation: Dissolve 2.0 grams of vanillin and 2.5 milliliters of acetaldehyde in 100 milliliters of ethanol.

4.6.1.2.1. Storage: Amber glass.

4.6.1.2.2. Expiration: Stock container: Three years

4.6.1.2.2.1. Use container: Three months

4.6.1.2.3. Lot number: Eight-digit format year/month/day/Duq/initials of preparer**.**

4.6.1.2.3.1. Example: 20120131DuqXXX

4.6.1.2.4. PQCC:

4.6.1.2.4.1. Reference material: Cannabis or Δ^9-Tetrahydrocannabinol.

4.6.1.2.4.2. Acceptable result: A violet blue color observed after the addition of acid and the violet color transfers to the chloroform layer, i.e., Positive.

4.6.2. Procedure

4.6.2.1. Place a small amount of sample in a culture tube or spot plate.

4.6.2.1.1. An evaporated petroleum ether or chloroform extract may be used. Record the preparation of the sample in the case file.

4.6.2.2. Add at least three drops of the Duquenois reagent and mix thoroughly.

4.6.2.2.1. The liquid may be decanted from the material and used to proceed. This may be needed with food products, residues, wet material or young plants.

4.6.2.3. Add an equal volume of concentrated hydrochloric acid and mix.

4.6.2.4. Observe any color changes.

4.6.2.5. Add three volumes of chloroform and mix.

4.6.2.6. Allow phases to separate and observe the color in the chloroform (bottom) layer.

4.6.2.7. Record results and any observations.

4.7. Sample Preparation for Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

4.7.1. Prepare sample(s) for GC-MS analysis utilizing an appropriate solvent and or extraction based on the sample to be analyzed.

4.7.1.1. For each batch of samples prepared, prepare a negative control in tandem using the same techniques, reagents, and materials as the sample extraction in approximately the same amounts.

4.7.2. Following sample extraction, evaporate the solvent with nitrogen gas.

4.7.3. Add approximately 0.25 ml - 0.50 ml of BSTFA with 1% TMCS to dried sample.

4.7.4. Cap samples and incubate at 70°C for 30 minutes.

4.7.5. Analyze sample(s) according to the Drug Chemistry Technical Procedure for Gas Chromatography/Mass Spectrometry.

5. Limitations

5.1. Not every cannabis exhibit contains every plant characteristic. The Drug Chemist shall identify and document those that are present.

5.2. Immature seedlings may not exhibit sufficient morphological characteristics for identification.

5.3. This procedure does not determine the quantitation or concentration of cannabinoids present in a suspected cannabis sample.

6. Safety

6.1. Use proper personal protective equipment when handling moldy cannabis/plant material.

7. References

7.1. *Marihuana Its Identification*. Washington, D.C.: U.S. Treasury Department Bureau of Narcotics, United States Printing Office, 1948.

7.2. *Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products.* New York: Laboratory and Scientific Section United Nations Office on Drugs and Crime, United Nations, 2009. https://www.unodc.org/documents/scientific/ST-NAR-40-Ebook_1.pdf

7.3. *North Carolina General Statutes* §90-87 (16) and §90-95(d)(4). https://www.ncleg.net/EnactedLegislation/Statutes/PDF/ByArticle/Chapter_90/Article_5.pdf

7.4. Bailey, Keith, M.A. and D. Phil. "The Value of the Duquenois Test for Cannabis – A Survey." *Journal of Forensic Sciences.* Volume 24, Issue 4 (October, 1979): 817-841.

7.5. Pitt, C.G. et. al. "The Specificity of the Duquenois Color Test for Marijuana and Hashish." *Journal of Forensic Sciences*. Volume 17, Issue 4 (Oct. 1972): 693-700.

8. Records

8.1. Prepared Reagent Log

Document Revision History		
Revision Date	Prepared By	Revision
11/6/2024	A. Abernethy	Document revised to reflect the agency name change from Raleigh/Wake City-County Bureau of Identification to Wake County Bureau of Forensic Services, effective December 1, 2024. Changed header and revision history format. No change to procedure content.