1.0 Purpose - This procedure specifies the required elements for the identification of plant material (not to include fungi) and plant material extracts.

2.0 Scope - This procedure applies to all plant material (not to include fungi) and plant material extract exhibits analyzed in the Drug Chemistry section of the Pitt County Sheriff's Office Forensic Services Unit.

3.0 Definitions

- **Hashish** - Common name for the extracted resin of marijuana.
- **Performance verification –** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check –** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **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.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Microscope(s) – stereo and/or polarizing
- Balance
- Gas Chromatograph/Mass Spectrometer (GC-MS)
- Laboratory coat
- Gloves
- Small culture tubes
- Reagent bottles and stock bottles (amber-colored preferred for Duquenois reagent)

4.2 Materials and Reagents

- Marijuana/Hemp or Hashish/THC/cannabinoid reference material
- Weigh vessel
- Acetaldehyde (ACS grade or higher)
- Vanillin (ACS grade or higher)
- Ethanol (ACS grade or higher)
- Concentrated Hydrochloric Acid (ACS grade or higher)
- Chloroform (ACS grade or higher)
- Hexane or Methanol (ACS grade or higher)
- Suspected plant material (not to include fungi)/plant material extract exhibit

5.0 Procedure

5.1 **Standards and Controls -** A primary or secondary reference material of marijuana/hemp and/or hashish shall be used for macro and microscopic comparison purposes.

5.2 **Sampling -** Plant material shall be sampled according to the Technical Procedure for Sampling.

5.3 **Stereo Microscopes**

5.3.1 New stereo microscopes shall be installed by an approved vendor according to the manufacturer's instructions, and documented in the Document Management (DM).

5.3.2 Maintenance of Stereo Microscopes

5.3.2.1 Stereo microscopes shall be serviced yearly by an approved vendor, and service visits documented in DM.

5.3.2.2 When a microscope has been placed out of service (e.g., maintenance, malfunction, leaving the direct control of the Laboratory), correct operation shall be verified by turning on the light source and focusing on a sample of plant material under the objective to ensure proper function has been returned.

5.3.2.3 Laboratory personnel shall examine the effect(s) if any, of a malfunction on analysis and implement the Laboratory Procedure for Corrective Action as required.

5.3.3 Operation of Stereo Microscopes

5.3.3.1 Switch on the light source. (Refer to the operator manual for location/description of specific parts mentioned below.)

5.3.3.2 Place the specimen slide on the stage.

5.3.3.3 Focus with the adjustments for the desired objective.

5.3.3.4 Move the exhibit around to view, adjusting the focus accordingly.

5.3.4 Application of Procedure on Evidence with stereo microscope

5.3.4.1 Plant material shall be weighed according to the Technical Procedure for Balances and reported with applicable measurement assurance.

5.3.4.2 Plant material shall be viewed macroscopically and microscopically to verify the presence of visually recognizable morphological characteristics.

5.3.4.3 Macroscopic and microscopic characteristics present in the exhibit shall be documented in the casenotes by checking the box beside the characteristic(s).

5.3.4.3.1 An indication of hairs implies the observation of both cystolithic and glandular hairs for that exhibit, unless otherwise noted.

5.4 Microscopic Examination of Marijuana/Hashish for Cystolithic Hairs Using Chloroform and the Polarizing Microscope

5.4.1 See Technical Procedure for Polarized Light Microscopy for details when this technique is needed for analysis.

5.4.2 Chloroform can be used to aid in the visualization of plant particles of marijuana/hemp or hashish.

5.4.2.1 Marijuana/hemp or hashish cystolithic hairs look like bear claws.

5.4.2.2 For frequently seen cystolithic hairs, the marijuana/hemp or hashish standard is not required to be run with each test sample.

5.4.3 Application of Procedure on Evidence with polarizing microscope

5.4.3.1 Add a drop of chloroform to the microscope slide. View to ensure no hairs are present.

5.4.3.1.1 If hairs are present, steps will be taken until no hairs are present. This includes, but is not limited to, obtaining a new microscope slide or new solvent.

5.4.3.2 Place a small sample of suspected hashish or marijuana/hemp onto the microscope slide and mix the chloroform with the material.

5.4.3.3 Observe under a relatively low magnification (approximately 10x). Record results of the microscopic examination in the casenotes.

5.4.4 Microscopic characteristics present in the exhibit shall be documented in the casenotes.

5.5 Macroscopic and Microscopic Characteristics of Marijuana/Hemp

5.5.1 Macroscopic characteristics that may be observed include:

- Upright stalk attains a height of 3-16 feet, average 4-6 feet.
- Stalk varies in diameter up to two inches, averages less than one half inch.

- Plant has compound palmate leaves with 5-11 leaflets (usually seven), and odd in number.
- Leaf is similar in shape to a hand.
- Leaflets are pointed at both ends and vary up to about six inches length and to about 1.5 inches in width.
- Leaves are green, brown-spotted, or brown in color.
- Distinction between male and female plants is difficult except at maturity.

  Male: flowers are very prominent; mature ones shed pollen profusely.
  Female: flowers are inconspicuous and are found hidden among the small leaves at the ends of the stalk and branches.

- 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 right angles.
- Plant has a characteristic odor.
- Seeds have a lacy, mottled appearance like a melon or turtle's back.
- Seeds are ovoid in shape, mottled in color and are greenish-yellow to brown.
- Seeds are enclosed in bulbs or pods (hulls).
- One main tap root up to eight inches long. Smaller branches from the main root.

5.5.2 Microscopic characteristics that may be observed include:

5.5.2.1 Leaves or leaflets

- Green, brown-spotted, or brown in color.
- Characteristically serrated.
- Veins end at sharp point of each serration or notch, best seen from the underside.
- Cystolithic hairs on upper side.
- Glandular hairs on underside.
- Effervescence with dilute hydrochloric acid.

5.5.2.2 Stems

- Fluted
- Branches appear immediately above each leaf.
- Hairs

5.5.2.3 Seeds (Fruit)

- Greenish-yellow to brown in color.
- Lacy, mottled appearance like a melon or a turtle's back.
- Ovoid in shape.
- Ridge around the greatest circumference.

- Inside similar to coconut meat.

5.5.2.4 Hairs

5.5.2.4.1 Cystolithic hairs

- Characteristic "warty" appearance; look like bear claws.
- Sphere of calcium carbonate at the base of the hair which effervesces in dilute hydrochloric acid.
- No plant which fails to show them can be marijuana/hemp.

5.5.2.4.2 Glandular hairs

- Wooly appearance; look like clubs with flattened, spherical heads.

5.5.2.5 Hulls (pods) - found on outside of seeds

- Green, brown or brown-spotted in color.
- Characteristically shaped.
- Cystolithic and glandular hairs on outer surface.

5.6 Duquenois-Levine (Modified) Color Test

5.6.1 This color test reacts with marijuana/hemp, hashish, and cannabinoids to produce a violet blue color that transfers to the chloroform layer.

5.6.2 **Standards and Controls –** Quality control checks shall consist of a negative check and a positive check. Both checks shall be acceptable (see below), and shall be recorded together as a quality control check in the Reagent Log stored in the Document Management System (DM).

5.6.2.1 Negative quality control checks shall be performed according to the procedure listed (see below) with no sample present.

5.6.2.1.1 Acceptable result is no significant color formation.

5.6.2.1.2 If color develops, steps shall be taken to ensure the culture tube is clean. Making new reagent and retesting with no sample present are further steps that can be taken to ensure no significant color develops.

5.6.2.2 Positive quality control checks shall be performed according to the procedure listed using the specified reference material (see below).

5.6.2.2.1 The result of the quality control check shall be recorded in the reagent log with the identification of the standard used and the results of the QC check.

5.6.2.3 Reagents may be prepared in any amount provided that the component ratios are kept constant.

5.6.2.4 Storage - Stock and use solutions shall be stored in closed containers. All stock bottles shall be stored in the refrigerator, and all use bottles kept on the countertop or under the hood, unless otherwise noted.

5.6.2.4.1 Expiration Dates - Stock bottles stored in the refrigerator have a three year expiration date. They shall be labeled as such.

5.6.2.4.2 For use bottles, the expiration date is three years unless specifically stated in the procedure.

5.6.2.5 For all stock and use bottles, rechecks will be performed at six month intervals.

5.6.2.5.1 For stock bottles that are not used directly, each time an aliquot is removed to prepare a use container, a QC check must be performed.

5.6.3 Application of Procedure on Evidence –

5.6.3.1 Duquenois (A) **-** Dissolve 2.0 grams of vanillin and 2.5 milliliters of acetaldehyde in 100 milliliters of ethanol.

5.6.3.1.1 Amber-colored use bottles shall be used to protect this reagent from light**.**

5.6.3.1.2 Use bottles have a three month expiration date if stored on the bench. If stored in the refrigerator, use (and stock) bottles shall have a three year expiration date, but shall be QC checked every six months.

5.6.3.1.3 Suggested Lot number format:
Year/month/day/Duq/initials of preparer**.**

5.6.3.2 Concentrated Hydrochloric Acid (B)

5.6.3.2.1 Prepare a (dropper) bottle of concentrated hydrochloric acid.

5.6.3.3 Chloroform (C)

5.6.3.3.1 Prepare a (dropper) bottle of chloroform.

5.6.3.4 Work instructions for the Duquenois-Levine (Modified) color test

5.6.3.4.1 Place a small amount of sample in a culture tube.

5.6.3.4.2 Add two to three drops of the Duquenois reagent (A).

5.6.3.4.3 Add at least an equal volume of concentrated hydrochloric acid (B) and observe any color changes.

5.6.3.4.4 Add at least two to three drops of chloroform (C) and agitate.

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

5.6.3.4.6 Record results in the case file if performing casework, or in the reagent log if performing a QC check.

5.6.3.4.7 QC check: Marijuana/hemp/THC produces a violet blue color after addition of the hydrochloric acid. For a positive result, this color shall transfer to the chloroform layer with shaking.

5.6.3.4.8 Results: Marijuana/hemp/THC, hashish, cannabinoids – violet blue color after addition of the hydrochloric acid, which extracts into the chloroform layer with shaking.

5.6.4 Limitations of the Duquenois-Levine (Modified) color test

5.6.4.1 For wet or fresh plant material, the color development may be hindered. In these cases, wash the wet or new plant material with the Duquenois reagent quickly and decant the reagent to a new culture tube. Proceed with addition of acid and chloroform as described in the procedure.

5.6.4.2 For old plant material, the color development may be hindered. In these cases, place the material in a culture tube. Cover with petroleum ether and let sit for approximately two minutes. Decant the petroleum ether to a clean culture tube. Evaporate petroleum ether on hot plate. (Set tube in a beaker for support if needed.) Proceed with addition of Duquenois reagent, acid, and chloroform, to the residue left from the petroleum ether wash.

5.6.4.3 If color formation is slow, a small amount of heat may be added to the plant material and Duquenois reagent to facilitate color development. (Careful use of a heat gun is suggested.)

5.6.4.4 For smoking devices and/or paraphernalia, the item may be washed with chloroform to remove the marijuana/hemp/THC residue. Duquenois-Levine reagent, and acid are then added to a portion of the chloroform wash as described in the procedure.

5.7 Minimum Criteria for Identification of Material with Botanical Features - For plant material that exhibits the characteristic botanical features of marijuana/hemp, the following examination is considered an acceptable minimum criteria for positive identification:

5.7.1 Physical (macroscopic and microscopic) to include at least one of the following combinations of microscopic characteristics:

5.7.1.1 Leaf/leaflets/leaf fragment(s) and hairs ***OR***
5.7.1.2 Stem(s) and hairs ***OR***
5.7.1.3 Seed(s) and hairs

AND

5.7.2 A positive Duquenois-Levine (Modified) color test. (See above)

AND

5.7.3 GC-MS analysis identifying either: Tetrahydrocannabinol and/or Cannabidiol.

5.7.3.1 Retention time match to Tetrahydrocannabinol (THC) and/or Cannabidiol (CBD) reference material shall be used if a Modified Duquenois-Levine color test was not possible due to sample size or sample matrix. (See the Drug Chemistry Technical Procedure for Gas Chromatograph/Mass Spectrometry (GC-MS)).

5.7.3.2 For GC-MS analysis of plant material, soak a small portion of the exhibit in chloroform (or other appropriate organic solvent). Filter the chloroform/solvent prior to placing it in a GC-MS vial.

5.7.4 Material identified by the criteria above shall be reported as:

5.7.4.1 "Plant material belonging to the genus *Cannabis* containing Tetrahydrocannabinol (THC)* and Cannabidiol (CBD)*. Concentration of cannabinoid(s) not determined." *as determined by the analysis

5.8 **Minimum Criteria for Identification of Material without Botanical Features -** For material that does not meet the characteristic botanical features of marijuana/hemp listed directly above, the following shall be required:

5.8.1 A positive Modified Duquenois-Levine color test shall be obtained if sample size allows. (See above.)

AND

5.8.2 GC-MS analysis identifying either: Tetrahydrocannabinol and/or Cannabidiol.

5.8.2.1 Retention time match to THC(s) and/or Cannabidiol Reference Material shall be used if a Modified Duquenois-Levine color test was not possible due to sample size or sample matrix. (See the Drug Chemistry Technical Procedure for GC-MS)

5.8.3 Material identified by the criteria in this section (positive Modified Duquenois-Levine and GC-MS) shall be reported as one of the following:

5.8.3.1 "Tetrahydrocannabinol and Cannabidol*. Concentration of cannabinoid(s) not determined." *as determined by the analysis

NOTE: See the US Sentencing Commission Guidelines Manual for the Federal definitions of Hashish and Hashish Oil. Should the exhibit qualify under either of those definitions, and there is an indication the case will be prosecuted federally, the addition of "Hashish" or "Hashish Oil" may be added to the end of the reporting statement listed above.

6.0 **Uncertainty of Measurement -** See the Technical Procedure for Balances and the Technical Procedure for Measurement Assurance.

7.0 **Limitations -** Not every marijuana/hemp exhibit contains every plant characteristic. The chemist shall identify and document those that are present. The current procedures at the Pitt County Sheriff's Office Forensic Services Unit do not allow for the differentiation of "marijuana" from "hemp", as defined in North Carolina General Statues.

8.0 **Safety -** Mold that grows on marijuana/hemp is an inhalation hazard. Precautions (such as the use of an N-95 particulate respirator) shall be taken when handling molded plant material.

9.0 **References**

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10.0 Records

- Case files
- Reagent Log

REVISION HISTORY		
CURRENT VERSION	EFFECTIVE DATE	SUMMARY OF CHANGES
1	2020/01/15	**Original Document –** Based on the Pitt County Sheriff's Office Forensic Services Unit Drug Chemistry Technical Procedure for the Identification of Marijuana, Version 3, Effective 2018/10/22.