1.0 Purpose - This procedure specifies the required elements for the identification of Marijuana as defined in NC General Statute §90-87 (16) and Hashish as defined in §90-95(d)(4).

2.0 Scope - This procedure applies to all cannabis exhibits analyzed in the Drug Chemistry section of the Pitt County Sheriff's Office Forensic Science 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) – basic and/or polarizing
- Balance
- Fume hood
- Eye protection
- Laboratory coat
- Gloves
- Small culture tubes
- Reagent bottles and stock bottles (amber-colored preferred for Duquenois reagent)
- Commercial Reagents
- Reference materials

4.2 Materials and Reagents

- Marijuana (or Hashish/THC) reference material
- Weigh vessel
- Modified Duquenois-Levine reagent
- Suspected cannabis exhibit

5.0 Procedure

5.1 Standards and Controls - A primary or secondary reference material of marijuana 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 Application of Procedure on Evidence

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

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

5.3.2.1 If the net weight of the item is less than five grams, and consists of hand-rolled cigarettes or partial hand-rolled cigarettes, the paper will be included in the weight recorded/reported with applicable measurement assurance. The evidence can be cut open to expose the plant material for viewing and analysis.

5.3.2.1.1 For purposes of sampling, one unit shall be analyzed and reported with the weight of the paper. All other units in the population shall be left unanalyzed and no weight reported.

Example:
Description
Plastic bag containing multiple hand-rolled cigarettes.

Results
One hand-rolled cigarette:
Marijuana – Schedule VI.
Net weight of paper and plant material - 0.30 (+/- 0.0X) gram (confidence level 99.7%).

Remaining material – No chemical analysis.

5.3.3 Macroscopic and microscopic characteristics present in the exhibit shall be documented on the worksheet by checking the box beside the characteristics.

5.3.4 Macroscopic characteristics:

- 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.
- 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.

5.3.5 Microscopic Characteristics

5.3.5.1 Leaves

- 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.
- Longer, sharper pointed (guard) hairs on underside.
- Effervescence with dilute hydrochloric acid.

5.3.5.2 Stems

- Fluted
- Branches appear immediately above each leaf.

5.3.5.3 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.

5.3.5.4 Hairs

5.3.5.4.1 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.

5.3.5.4.2 Glandular hairs

- Glandular hairs have a wooly appearance; look like clubs with flattened, spherical heads.

5.3.5.4.3 Guard hairs

- Longer and fine.

5.3.5.5 Hulls (pods) - found on outside of seeds

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

5.3.6 Duquenois-Levine (Modified) Color Test

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

5.3.6.2 **Standards and Controls –** Quality control checks of all reagents shall consist of a negative check and a positive check. Both checks shall be acceptable according to the procedure listed for each reagent, and shall be recorded together as a quality control check in the Reagent Log stored in the Document Management System (DM).

5.3.6.2.1 Negative quality control checks shall be performed according to the procedure listed with no sample present.

5.3.6.2.1.1 Acceptable result is no significant color formation.

5.3.6.2.1.2 If color develops, steps shall be taken to ensure the spot plate is clean. Making new reagent and retesting with no sample present are further steps that can be taken to ensure no significant color develops prior to introduction of the sample.

5.3.6.2.2 Positive quality control checks shall be performed according to the procedure listed for each reagent using the specified reference material. See each procedure for acceptable results.

5.3.6.2.2.1 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.

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

5.3.6.2.4 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 in the procedure.

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

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

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

5.3.6.2.5.1 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.

5.3.6.3 **Application of Procedure on Evidence** –
Duquenois (A) **-** Dissolve 2.0 grams of vanillin and 2.5 milliliters of acetaldehyde in 100 milliliters of ethanol.

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

5.3.6.3.2 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.

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

5.3.6.4 Concentrated Hydrochloric Acid (B)

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

5.3.6.5 Chloroform (C)

5.3.6.5.1 Prepare a (dropper) bottle of chloroform.

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

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

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

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

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

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

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

5.3.6.6.7 QC check: Marijuana 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.

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

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

5.3.6.7.1 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.

5.3.6.7.2 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.

5.3.6.7.3 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.)

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

5.3.7 Minimum acceptance criteria for the identification of Marijuana shall include:

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

AND

5.3.7.2 A combination of at least the following microscopic characteristics:

- Leaf/leaf fragment(s) and hairs ***OR***
- Stem(s) and hairs ***OR***
- Seed(s) and hairs

5.3.8 For material that does not meet the minimum acceptance criteria listed directly above, the following shall be required:

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

AND

5.3.8.2 GC-MS analysis

5.3.8.2.1 Retention time match to THC Reference Material shall be used if a Modified Duquenois-Levine color test was not possible due to sample size.

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

- Tetrahydrocannabinol (THC) – Schedule VI
- Hashish – Schedule VI.
- Resinous material containing Tetrahydrocannabinol (THC) – Schedule VI.

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

6.0 Limitations - Not every marijuana exhibit contains every plant characteristic. The chemist shall identify and document those that are present.

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

8.0 References

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9.0 Records

- Case files
- Reagent Log

REVISION HISTORY		
CURRENT VERSION	**EFFECTIVE DATE**	**SUMMARY OF CHANGES**
1	2017/11/14	Original Document.
2	2018/04/01	Scope – Updated "Illicit Drugs Discipline" to "Drug Chemistry Section
3	2018/10/22	**Equipment, Materials and Reagents** – Added materials associated with Duquenois-Levine color test, and removed reference to Technical Procedure for Preliminary Color Tests. **5.3.2.1.1** – Added confidence level to results example. **5.3.6.2** Added Standards and Controls for Duquenois-Levine color test reagent. **5.3.6.3.2** Clarified expiration dates bench/refrigerator. **5.3.8.1** Removed reference to Technical Procedure for Preliminary Color Tests. **References** - Transferred Nakamura reference from Technical Procedure for Preliminary Color Tests to this procedure. **9.0** Added reagent log