6 MARIJUANA AND HASHISH OIL

6.1 Introduction:

6.1.1 Marijuana is neither a "controlled substance" nor is it "scheduled" under Virginia Law. However, it is defined and covered under separate sections of the Drug Control Act of Virginia and has associated penalties.

6.1.2 Cannabis (marijuana) contains tetrahydrocannabinol (THC) in both the male and female plants. THC is found in all parts of the plant in varying concentrations.

6.1.3 Hashish oil is an oily extract containing one or more cannabinoids with little, if any, plant material present and containing 12% or more THC. It is controlled in Schedule I.

6.2 Macroscopic Identification:

6.2.1 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 stalks.

6.2.2 Due to the compressed or mutilated nature of many samples, many of these characteristics may not be discernable.

6.2.3 Positive macroscopic examination results may be recorded in the analytical notes by the use of an abbreviation of positive for characteristics of Marijuana (e.g., pos. char. mj), a plus (+), or a plus circled (⊕)). A result is considered positive when sufficient characteristics are observed and are specified in the case notes. Negative observations may be recorded in a similar fashion.

6.3 Microscopic Identification:

6.3.1 View the sample at varying magnifications (approximately 10 – 40x) using a stereomicroscope.

6.3.2 Cystolithic hairs are unicellular, “bear claw” shaped hairs with a cystolith of $CaCO_3$ at the base. They are found in greatest abundance on the upper side of the leaf with longer covering hairs on the underside.

6.3.3 Seeds are coconut shaped, veined (with lacy markings) and have a ridge around the circumference.

6.3.4 The observation of the presence of appropriate cystolithic hairs or characteristic seeds is sufficient for a positive test. The observation of additional characteristics is considered supportive. Positive microscopic examination results will be recorded in the analytical notes by the use of an abbreviation of positive for characteristics of Marijuana (e.g., pos. char. mj), a plus (+), or a plus circled (⊕). A result is considered positive when sufficient characteristics are observed. Negative observations will be recorded in a similar fashion.

6.4 Thin Layer Chromatography (TLC):

6.4.1 Extract sample into a suitable solvent (e.g., hexane, petroleum ether or methanol) and spot on plate. Spot standard next to sample(s).

6.4.2 TLC plates should be silica gel or equivalent, sufficient to resolve the three major cannabinoids listed in section 6.4.4.4.

6.4.3 Mobile Phase: 4-8% diethylamine in toluene

6.4.4 Visualization Sprays

6.4.4.1. Fast Blue B Salt (Tetrazotized o-dianisidine zinc chloride salt)

6.4.4.2. Fast Blue BB Salt (4-benzoylamino-2,5-diethoxy-benzenediazonium chloride hemi [zinc chloride] salt)

6.4.4.3. Reagent preparation is listed in the Thin Layer Chromatography section of this manual. (see 9.5.6)

6.4.4.4. Results:

- The three major cannabinoids migrate and develop in the following order:
  - Top spot - Cannabidiol – orange
  - Middle spot - Tetrahydrocannabinol (Δ^9-THC) - red
  - Lower spot - Cannabinol – purple
- A red spot at the origin may be present in unburned marijuana due to cannabinolic acid(s).

6.4.5 Samples will be screened for the presence of other commonly encountered drugs such as cocaine or PCP by either overspraying with acidified iodoplatinate or by running a separate plate in an appropriate drug bath (e.g., TLC1 or TLC2.) (see 9.4)

6.4.6 Specific solvent systems and developing sprays utilized in casework will be denoted in the analytical case notes. Positive TLC results may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) along with the standard used in the comparison. A result is considered positive when the distance traveled and the reaction with the visualization methods compare favorably with a standard. It is not necessary for each of the three major cannabinoids to be present for the results to be considered positive. Negative reactions may be recorded in a similar fashion.

6.5 Duquenois-Levine:

6.5.1 Extract sample into a suitable solvent (e.g., hexane, petroleum ether or methanol). If a large amount of solvent is used, most of it must be evaporated.

6.5.2 Add approximately equal amounts of Duquenois reagent and concentrated HCl to extract. A positive reaction to the Duquenois portion is a blue/purple color.

6.5.3 Add sufficient $CHCl_3$ to form two discernable layers and mix. For a positive reaction to the Levine portion of the test, the bottom layer turns pink/purple in the presence of THC or other cannabinoids.

6.5.4 Reagent preparation is listed in the Color Test section of this manual. (see 8.2.6)

6.5.5 The Rapid Duquenois-Levine Procedure

6.5.5.1 Place a small amount of plant material in a culture tube, add Duquenois reagent and concentrated HCl in approximately equal proportions. Observe a blue/purple color. Add $CHCl_3$ and observe extraction of pink/purple color into the $CHCl_3$ layer.

6.5.6 Results must be recorded in the case notes with a single plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) indicating a positive result in both steps or the colors of each step may be noted. Negative reactions may be recorded in a similar fashion.

6.6 Gas Chromatography/Mass Spectrometry (GC/MS):

6.6.1 GC/MS should be performed if the results from any of the prior three tests are inconclusive.

6.6.2 Retention time data is not required.

6.7 Hashish Oil:

6.7.1 Analytical Scheme

6.7.1.1 Weigh or approximate the volume of the material.

6.7.1.2 Dilute with appropriate solvent and perform TLC as listed for marijuana. The presence of additional cannabinoids will confirm that the THC is most likely from the marijuana plant.

6.7.1.3 Perform the Duquenois-Levine test.

6.7.1.4 Dilute with appropriate solvent and run on GC/MS to confirm the presence of THC.

6.7.1.5 Quantitate THC using method below.

6.7.2 THC Quantitation

6.7.2.1 Reagents

- n-Hexane: High purity
- Ethyl Alcohol: (95%) USP Grade
- Delta-9-Tetrahydrocannabinol: USP Grade 10 mg/mL in EtOH)
- n-Triacontane: 99% pure or greater.

6.7.2.2 Internal Standard Solution

6.7.2.2.1 Prepare a sufficient volume to dilute the THC standard solution and all samples.

6.7.2.2.2 Prepare a 1 mg/mL solution of n-triacontane in n-hexane in the appropriate volumetric flask.

6.7.2.3 THC Standard Solution

Quantitatively transfer 1 mL of ethanol containing 10 mg THC from the ampoule to a 10 mL volumetric flask using 95% EtOH. Dilute to volume with 95% EtOH and mix thoroughly. This results in a 1 mg/mL solution. Pipette 2 mL of this solution into a culture tube. Evaporate to dryness. Pipette 3 mL of internal standard solution into the culture tube and mix thoroughly. This results in a 0.667 mg/mL solution of THC in internal standard solution.

6.7.2.4 Sample Preparation:

Weigh 10-20 mg of sample into a test tube and add 5 mL internal standard solution.

6.7.2.5 Suggested Columns

- 100% methylsilicone (HP1 or equivalent) capillary (15 m, 0.25 mm I. D., 0.25 µm film thickness)
- 5% phenylmethylsilicone (HP5 or equivalent) capillary (15 m, 0.25 mm I. D., 0.25 µm film thickness)

6.7.2.6 Oven Temperatures: Approximately 240 - 260°C

6.7.2.6.1 THC elutes before n-triacontane.

6.7.2.7 THC acid converts to THC upon heating in the injection port, causing errors in the quantitation if present. To avoid this, after quantitating, add 1 mL 1.5% aqueous NaOH solution to the vial, shake vigorously for 30 seconds and let settle. Pipette the hexane into another vial and re-inject. If the values

are approximately the same, no acid was present. Large differences would be expected if a significant red spot at the origin was noted during the TLC test.

6.7.2.7.1 If the results are more that 10% different, extract the standard and each sample with base and requantitate.

6.7.2.8 Store THC and internal standard solutions closed in the refrigerator.

6.7.3 Reporting:

6.7.3.1 Hashish oil will be reported as currently defined by the Code of Virginia. Current example: Hashish oil (Schedule I), which contains ___% tetrahydrocannabinol by weight.

6.8 References:

6.8.1 C.G Pitt, R.W. Hendron and R.S. Hsia, "The Specificity of the Duquenois Color Test for Marijuana and Hashish", *Journal of Forensic Sciences*, 1972, Volume 17, No. 4, Pages 693-700.

6.8.2 G.R. Nakamura, " Forensic Aspects of Cystolithic Hairs of Cannabis and Other Plants", *Journal of the Association of Official Analytical Chemists,* Volume 52, No. 1, 1969, Pages 5- 16

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