Marijuana
Standard Operating Procedures

1.0.0 Background

Marijuana (Cannabis Sativa) has been used for its sedative, euphoriant and hallucinogenic properties for over 3000 years. Written references to it date back to 2700 BC. It is primarily smoked but can be taken orally. The active compound, delta-9-tetrahydrocannabinol (THC) is most concentrated in the resin that is obtained from the flowers of the female plant. It is imperative that the analyst be familiar with the current Idaho code as it pertains to the legal definition of marijuana.

2.0.0 Scope

The following analytical procedures are used to confirm the presence of marijuana in plant material and residue samples. The procedure is composed of a series of tests, none of which by themselves are specific for marijuana or THC, but taken in combination are considered specific for the presence of marijuana or its resins. GC/MS is not routinely applied to marijuana analysis but may be used and is considered specific for THC.

3.0.0 Equipment and Reagents

3.1.0 Stereo microscope.
3.2.0 Thin layer chromatography tank and plates.
3.3.0 Aqueous Fast Blue BB solution. (Fast Blue B salt may be used as a substitute)
3.4.0 Petroleum ether, hexane, diethyl ether, benzene, methanol, toluene, and chloroform.
3.5.0 GC/MS and analytical software.

4.0.0 Solvent Extraction

4.1.0 Plant material
- 4.1.1 Place approx. 0.1g of plant material in test tube.
- 4.1.2 Cover with petroleum ether or hexane.
- 4.1.3 Use extract for thin layer and/or modified Duquenois-Levine.
- 4.1.4 Retain small amount of unused solvent as blank.

4.2.0 Residues
- 4.2.1. Flush pipe or item(s) containing suspected residue with petroleum ether or hexane and collect solvent in test tube (item(s) may also be swabbed).
- 4.2.2. Use extract for thin layer and/or modified Duquenois-Levine.
- 4.2.3. Retain small amount of unused solvent as blank.

5.0.0 Microscopic Examination

5.1.0 Plant material is examined using a stereo microscope for the following characteristics:
- 5.1.1 Cystoliths and/ or Cystolithic hairs – Small "bear claw" shaped hairs with bases of calcium carbonate. The cystoliths and hairs are located on the topside of the leaf or leaf- fragment.

5.1.2 Unicellular hairs – Fine hairs located on the underside of the leaf or leaf-fragment.

5.2.0 Seeds are examined using a stereo microscope for the following characteristics:
- 5.2.1 Veined shell.
- 5.2.2 Ridged edges.
- 5.2.3 Point on one end and dint on the end of plant attachment.

6.0.0 Thin Layer Chromatography

6.1.0 Spot a small amount of petroleum ether/hexane extract onto a thin layer plate along side of a marijuana standard and a solvent blank.

6.2.0 Develop the plate using one or more of the following mobile phases:
- 6.2.1 Hexane/diethyl ether 4:1 (petroleum ether may be substituted for hexane).
- 6.2.2 Chloroform.
- 6.2.3 Benzene
- 6.2.4 Petroleum ether/methanol 95:5 (if PCP is suspected)
- 6.2.5 Toluene

6.3.0 Visualize by spraying the plate with Fast Blue BB salt solution.

6.3.0 Compare results of unknown to those of standard. Photocopy the plate for the case file.

7.0.0 Modified Duquenois-Levine

7.1.0 In a test tube containing a portion of the evaporated petroleum ether/hexane extract, mix 2-10 drops of Duquenois reagent and an equal amount of concentrated HCl.

7.2.0 Let stand ½ to 3 minutes and observe color change.

7.3.0 Add chloroform.

7.4.0 Observe if the purple color transfers into chloroform layer. * Note* Transferring the solution from step 7.2.0 into a clean test tube before the addition of chloroform will decrease the color interference from chlorophyll.

8.0.0 Results and Reporting

A positive test shall be defined as the following:

8.1.0 Microscopic
- 8.1.1 Observation of cystolithic and unicellular hairs on the same leaf and/or the presence of characteristic seeds.

8.2.0 Thin Layer
- 8.2.1 Presence of a scarlet spot with migration distance consistent with the scarlet THC spot of the standard.
- 8.2.2 Negative blank.

8.3.0 Modified Duquenois-Levene
- 8.2.1. A purple* color developing after the addition of the HCl (*color may very from blue to reddish purple depending on the sample).
- 8.2.2. Transfer of the color into the organic layer after the addition of chloroform.

A positive result shall be defined as the following:

8.4.0 Positive microscopic, single TLC system, and modified Duquenois-Levine.
8.4.1 Report as "contains marijuana. Schedule I, non-narcotic".
8.5.0 Negative microscopic. Positive modified Duquenois-Levene and two positive TLC systems.
8.5.1 The conclusion should contain the words "contains, marijuana, and resins."

9.0.0 Germination

Marijuana seeds without THC are only controlled if they are fertile. The germination test should only be performed if it has been determined that the seeds do not contain THC. *Note* In determining the presence of THC, soaking the seeds for up to thirty minutes in petroleum ether /hexane, does not effect germination rates.

9.1.0 Wrap a minimum of 10, to a maximum of 100 seeds, in a moist paper towel and place in a covered container. The container is then placed in a safe place for 14 days.
9.2.0 Check seeds daily making sure they do not dry out. Also watch out for mould.
9.3.0 Report how many seeds sprouted as a percentage of the original total.

10.0.0 GC/MS Confirmation

10.1.0 Extract sample as in section 4.0.0
10.2.0 Run extract according to GC/MS SOP along with a known standard containing THC.
10.3.0 Compare retention time and ion chromatograph of sample with THC standard.
10.4.0 Report positive results using the words "Contains marijuana. Schedule I, non-narcotic."