1. Introduction

1.1 Plant Material

This procedure is designed to aid in the identification of marijuana (Cannabis sativa L) when vegetative material has been submitted to the laboratory for analysis. Due to the 2018 Farm Bill, the difference between industrial hemp and marijuana was based on the amount of THC within dried plant material. Industrial hemp and marijuana both are from the Cannabis Sativa plant and only differ based on cultivars.

Cannabis plant material can also contain cannabinoid chemicals such as cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA), delta-8-tetrahydrocannabinol (Δ^8-THC), and others. Morphological characteristics and microscopic observations within Cannabis vegetative material may be observed and their results used within the identification process of marijuana.

1.2 Manufacturer Hemp Products

This method will be used to gather further information regarding the approximate amount of delta9-THC in manufacturer hemp products. These products cover a wide range of matrices.

1.3 State and Federal Laws

The reporting criteria was established based on the 2018 Farm Bill and Connecticut Public Act No. 24-79 (and supplemental statutes).

2. Scope

This procedure can be used for the analysis of submitted specimens for the determination of the amount of THC present. This procedure utilizes semi-quantitative measures. Since THCA readily converts to THC upon heating and ingestion, when THC is identified then it is considered 'total THC content' (both THC and THCA).

3. Principle

3.1 Plant Material

The amount of delta-9-tetrahydrocannabinol (THC) present within case samples is determined by solvent extraction followed by gas chromatography/mass spectrometry (GC/MS). Marijuana is identified by using a combination of visual examination, microscopy, and instrumental analysis of extract solutions. For differentiation between hemp and marijuana, a decision point of 1% THC is used, which is well-above the legally defined 0.3% THC level. The ratio of delta9-THC: delta9-THC-d3 peak area within the sample will be compared to the ratio in the calibrator solution. The 1% THC decision point is an administratively defined cutoff concentration that is used to discriminate

between positive and negative results and allows an acceptable margin to be in place for reporting results.

3.2 Manufacturer Hemp Products

The amount of delta-9-tetrahydrocannabinol (THC) present within case samples is determined by QuEChERS extraction followed by gas chromatography/mass spectrometry (GC/MS). The ratio of delta9-THC: delta9-THC-d3 peak area within the sample will be compared to the ratio in the calibrator solution. For determination of a moderate-THC hemp product, a decision point of 1mg THC per dosage is used, which is well-above the legally defined 0.5mg THC level. The 1mg THC decision point is an administratively defined cutoff concentration that is used to discriminate between positive and negative results and allows an acceptable margin to be in place for reporting results.

4. Specimens

4.1 Plant Material

This procedure is used for the analysis of vegetative (or plant-like material (PM)) specimens to identify the presence of marijuana within submitted specimens. However, if non-vegetative specimens are submitted where enough vegetative material can be physically isolated (e.g., visible residue within a burnt cigarette, visible residue within a pipe/bong, plant-like material within food product), then this procedure can be used for such analyses.

4.2 Manufacturer Hemp Products

This procedure is limited to the analysis of gummies to determine the THC content per dosage unit and per container.

5. Equipment/Materials/Reagents

5.1 General laboratory glassware

5.2 Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with caps)

5.3 Autosampler vials (GC/MS grade or equivalent)

5.4 Balance (e.g., Top-loading, analytical, or bulk scale)

5.5 Microscope (MiScope® or equivalent)

5.6 PTFE Syringe Filters (or equivalent)

5.7 QuEChERS Extraction Salt Kits (Restek Cat. No. 25847 or equivalent)

5.8 Digital camera

5.9 Gas Chromatograph/Mass Spectrometer with a 30-meter DB-5MS column (or equivalent)

5.10 Vortex mixer

5.11 Centrifuge

5.12 Sonicator

5.13 Automatic pipettes (with disposable tips)

5.14 Acetonitrile (ACN; Reagent grade or equivalent)

5.15 Methanol (MeOH; $CH_3OH_{(l)}$, Reagent grade or equivalent)

5.16 Certified Reference Material (CRM) Solutions

5.16.1 THC (1 mg/mL) in MeOH (or equivalent)

5.16.2 THC-D_3 (1 mg/mL) (Cayman Chemical, Cerilliant, Lipomed, or equivalent)

6. Controls and Internal Standards

6.1 Controls

Positive/Negative Controls:
When applicable, store all materials according to their manufacturer's recommendations. If purchased, the stabilities of materials should be determined by the manufacturer. Appropriate positive and negative controls will be extracted and/or analyzed contemporaneously with each assay or batch. When certain reference standards are not available then consult the appropriate FSE2 (or higher) for guidance and whether certain standards can be analyzed separately from evidentiary samples.

Note: Volumes for reagent preparations can be adjusted using appropriate ratios in order to account for the number of samples that are to be extracted within specific batches.

6.1.1 Plant Material

6.1.1.1 Negative Control:

Any vegetative material that does not contain cannabinoid compounds may be used (e.g., tea, tobacco).

6.1.1.2 Positive Control:

6.1.1.2.1 Known marijuana vegetative material

6.1.1.2.2 Known hemp vegetative material

6.1.2 Manufacturer Hemp Product

6.1.2.1 Negative Control:

Any matrix-matched product that does not contain cannabinoid compounds may be used.

Note: An attempt will be made to match the matrix, but in some cases exact matrix matches may not be possible.

6.1.2.2 Positive Control:

Known moderate-THC hemp product

6.2 Internal Standards (ISTD)

6.2.1 Plant Material {Δ^9-THC-D_3; 0.1 mg/mL; Total Volume = 10 mL}

6.2.1.1 Pipette 1mL of a 1 mg/mL THC-D_3 solution into a 10mL volumetric flask. Fill to line using methanol. Cap and mix

6.2.1.2 This solution is stable (when stored in a freezer) for one year from its last verification or until the expiration date of the THC-D_3 CRM has been reached, whichever is earliest.

6.2.1.3 Alternatively, a 0.1 mg/mL certified reference material may be purchased.

6.2.2 Manufacturer Hemp Product

Follow the same procedure as Plant Material, using acetonitrile as the diluent instead of methanol.

6.3 Calibrator Solution {Δ^9-THC + Δ^9-THC-D_3; 0.01 mg/mL; Total Volume = 1 mL}

6.3.1 Plant Material

6.3.1.1 Pipette 10 µL of a 1 mg/mL Δ^9-THC reference standard into a test tube or autosampler vial.

6.3.1.2 Add 100 µL of 0.1 mg/mL internal standard solution or 0.1 mg/mL THC-D_3 certified reference material.

6.3.1.3 Add 890 µL of methanol and mix.

6.3.1.4 Prepare fresh.

6.3.2 Manufacturer Hemp Product

Follow the same procedure as Plant Material, using acetonitrile as the diluent instead of methanol.

Note: This gives a Δ^9-THC-D_3 concentration of 0.0099 mg/mL which is not exactly the same as the Δ^9-THC concentration, but the difference in deuterated internal standard concentration of 0.0099 mg/mL instead of 0.01 mg/mL is negligible, especially when the procedure is differentiating 1% THC from 0.3% THC.

7. Sample Preparation

7.1 Plant Material

7.1.1 Ensure the vegetative material is dry.

7.1.1 Unless moisture is visible and able to be transferred to paper-like material (e.g., brown paper, Kimwipe®, paper towel), samples will be considered to be in a dry state.

7.1.2 If plant-like samples are found to not be in a dry state then the appropriate FSE2 and management will be consulted to come up with a resolution prior to analysis.

7.1.2 Take a sample of the vegetative material (evidence and control samples).

7.1.3 For evidence samples only: perform a microscopic examination (e.g., using the MiScope®).

7.1.3.1 The MiScope® is a combination microscope and digital camera. The magnification of this device is about 40-140X. It connects directly into a computer and allows digital images to be obtained. Place a small amount of plant material on a clean piece of paper, position the MiScope® over the material, and use the zoom feature to magnify the area of interest.

7.1.3.2 Note/record any intact vegetative substance for the presence of cystolithic and/or glandular hairs. The shorter, bear claw'-shaped trichomes are the cystolithic-like trichomes, while the glandular trichomes often contain beads of resin on the ends.

7.1.3.3 When digitally capturing images each picture must include at least the date, case number, item number, and initials of the analyst capturing the image (this can be either within the image or within the filename). The image can be stored electronically within LIMS or printed within the case file.

7.1.4 Homogenize and grind vegetative material with mortar and pestle.

7.1.5 Individually weigh and isolate 50.0 mg +/- 1.0 mg of homogenized vegetative material (evidence; controls), place into a properly labeled test tube, and cap.

7.1.6 Individually add 0.5 mL of methanol to the vegetative material, vortex-mix for 30 seconds, and allow the extracts to sit for approximately 15 minutes.

7.1.7 Centrifuge the extract solutions (or filter through pre-rinsed filters), isolate the liquids in properly labeled test tubes/autosampler vials or similar storage container, and cap.

Note: If the vegetative materials within the above step contained 1% THC, then the resulting extract solutions would contain 0.1 mg/mL of THC. Such samples will be diluted to 0.01 mg/mL of THC in the next step (corresponding to the 0.01 mg/mL THC-D_3 IS and to the 0.01 mg/mL THC Calibrator Solution).

7.1.8 Individually dilute the extracts 1:100 by transferring 10 µL of each sample's extract solution into properly labeled autosampler vials, add 100 µL of internal standard solution or 100 µL of 0.1 mg/mL THC-D_3 certified reference material and add 890 µL of methanol. Cap and vortex-mix for about 30 seconds.

7.1.9 Analyze each solution (evidence; controls) using GC/MS. When necessary (e.g., identification of other cannabinoids), analyze appropriate reference standard controls.

7.2 Manufacturer Hemp Product

7.2.1 Weigh the whole gummy and record the weight for the decision criteria.

7.2.1.1 If the quantity and/or weight of the gummy is insufficient for analysis. Consult the appropriate FSE2 (or higher) for guidance.

7.2.2 Weigh 1.000 ± 0.005 grams from the middle of an infused gummy (cut into four pieces and use middle to avoid sugar sanding and waxy/oily coating) and chop very finely with scissors. Place at the bottom and sides of a 50mL PP conical-bottom tube using a stainless-steel spatula.

7.2.3 Add 10 mL of deionized water and cap. Place the tube in a sonication bath at approximately 60°C for approximately 10 minutes. Vortex for one minute.

7.2.4 Add 10 mL of acetonitrile and cap. Place the tube in a sonication bath at approximately 60°C for approximately 10 minutes. Vortex for one minute.

7.2.5 Add the contents of a QuEChERS extraction kit. Immediately shake for 10 seconds manually to avoid clumping. Open cap to degas, then tighten cap again. Vortex for 1 minute. Centrifuge the tube at 3,600 rpm minimum for 5 minutes at room temperature.

7.2.6 Filter the acetonitrile supernatant with PTFE filter. Transfer 900µL of the resulting solution to an autosampler vial, add 100µL of internal standard solution, cap, and vortex.

8. Setting-up Instrument with Samples

8.1 Ensure the appropriate instrumental quality assurance/quality control (QA/QC) procedures were performed. The instrument must have passing QA/QC results prior to use for casework. Analyze the THC calibrator solution for an additional QA/QC performance evaluation. Properly record and retain all QA/QC evaluation documents.

8.2 Prepare the instrumental sequence and enter the samples in the appropriate order.

8.2.1 Negative controls will be analyzed prior to evidentiary samples.

8.2.2 Blank samples will be analyzed in-between evidentiary samples in order to evaluate for carryover. These blanks shall be analyzed both at the beginning and at the end of each sequence.

8.2.2.1 Blank samples contain the diluent solvent only – no internal standard

8.2.2.2 Due to the variable nature of manufacturer products, it is highly recommended to run multiple blanks after the unknown. These blanks may include a combination of both acetonitrile and hexanes. Keep in mind that the blank ran immediately before the next unknown should always be acetonitrile.

8.2.3 The calibrator solution can be analyzed multiple times throughout the sequence.

Note: If the calibrator solution is analyzed in-between evidentiary samples, then the latest sample will be used to evaluate the previously analyzed samples as to whether their concentrations were greater or less than the calibrator solution.

8.3 Place the labeled autosampler vials in the appropriate order within the instrument.

8.4 Save the sequence with the day's date described in the filename. Ensure that the case number information, operator and instrument name are recorded within each sample description of the sequence.

8.5 Sequence Verification:

8.5.1 Ensure the sequence is set so that the instrumental method will be saved with each data file.

8.5.2 Generate the sequence list.

8.5.3 Check that the physical placement of the autosampler vials and the vial positions within the instrument's sequence list match.

8.5.4 Once the autosampler vial check has been completed, the sequence should be initialed and dated.

8.6 Verify and print the appropriate instrumental method that the sequence is using and include both the method and the sequence printouts with the eventual data printouts.

8.7 Within the autosampler tray ensure that appropriate wash vials contain the correct solvents and are full. Ensure that waste vials are empty.

8.8 Begin the sequence and analyze the samples.

9. Instrumental Parameters

For specific parameters, see method printout(s) contained within appropriate instrument binders (or electronically).

10. Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), fragmentation pattern and qualified ion ratios (mass spectrometric characteristics), and other characteristics are used as the basis for detection and identification. In most cases, all the criteria below should be met to identify the appropriate analyte(s).

10.1 Microscopy (Plant Material Only)

10.1.1 Marijuana and hemp will both have cystolithic-like trichomes present on their leaf fragments – usually on the upper surface of the leaves.

10.1.2 The presence of cystolithic-like trichomes on samples will support the determination of the vegetation as cannabis plant material.

10.1.3 Marijuana will not be identified without the observation of cystolithic-like trichomes being present on an item of evidence being analyzed.

10.1.4 Glandular trichomes should be present on cannabis vegetation but these hairs may be absent.

Note: The term cystolithic-like is used instead of cystolithic because it has not been scientifically proven that the trichomes contain calcium carbonate.

10.2 Chromatography

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics (e.g., reasonable peak shape, width, and resolution). For a chromatographic peak to be acceptable in a sample, it should compare favorably to the chromatographic peak(s) within a known sample, which has been analyzed on the same system and in the same analytical timeframe.

10.2.1 Retention Time (RT):

The retention time of a peak of interest should be within 0.1 minute of the retention time of a reference standard (i.e., calibrator or positive control reference standard).

10.3 Mass Spectrometry

Ion ratios should compare favorably to ion ratios of an extracted calibrator or positive control reference standard at a comparable concentration. Generally, ion ratios are within the limits as specified within the Section's procedure related to mass spectral comparisons.

10.4 **Sequence Acceptance**

For a sequence to be acceptable:

10.4.1 No analytes of interest will be detected within the Negative Control.

10.4.2 Significant carryover will be brought to the attention of the appropriate Lead Examiner (or higher) to determine if evidentiary samples have been negatively impacted. If so, re-analysis will occur, and sample re-extraction may be necessary. Appropriate case documentation will accompany these instances within affected case files to record events.

10.4.2.1 Significant carryover occurs when the signal intensity for an analyte peak is ten (10) times (or more) greater than the intensity from any carryover peaks which were present in just prior to the sample (e.g., Blanks or Negative Controls).

10.5 **Reporting Results using Instrument Software**

10.5.1 A one-point calibration line is generated by plotting data from the chromatographic area ratio of the calibration solution versus concentration.

Force the one-point calibration graph through the origin. This can be done by using the force-through origin setting within software.

10.5.2 Plant Material:

10.5.3.1 The chromatographic area ratio of analyte(s) from samples will be equal to or greater than those from the calibrator solution for the analyte(s) to be reported.

10.5.4 Manufacturer Hemp Products:

10.5.4.1 The chromatographic area ratio of analyte(s) from samples will be equal to or greater than the weight of the sample for the analyte(s) to be reported.

10.6 **Reporting Qualitative Results using Manual Calculation**

10.6.1 Plant Material

10.6.1.1 When the THC: THC-D_3 peak area ratio within a sample is greater than or equal to the THC: THC-D_3 peak area ratio within the THC calibrator solution, then the amount of total THC will be determined to be greater than 1.0%.

10.6.1.2 If the THC: THC-D_3 peak area ratio within a sample is less than the THC: THC-D_3 peak area ratio within the THC calibrator solution, then the amount of total THC will be designated as being less than 1.0%.

10.6.2 Manufacturer Hemp Product

10.6.2.1 When the THC: THC-D_3 peak area ratio within a sample is greater than or equal to the weight of the gummy, then the amount of total THC will be determined to be greater than 1mg per dosage unit.

10.6.1.2 If the THC: THC-D_3 peak area ratio within a sample is less than the weight of the gummy, then the amount of total THC will be designated as being less than 1mg per dosage unit.

Note: Any instances where the ratios are questionable and/or the decision process is not straightforward shall involve the appropriate Lead Examiner (or higher).

11. Calibration

This procedure is mainly qualitative and does have a pseudo-quantitative component.

12. Limitations

11.1 Plant Material

11.1.1 Cut-Off/Decision Point is 1% THC

11.1.2 Reporting:

11.1.2.1 The results on the final report will be “delta9-THC content is above 1%” or “delta9-THC content is below 1%”.

11.2 Manufacturer Hemp Product

11.1.1 Cut-Off/Decision Point is 1mg THC.

11.1.2 Reporting:

11.1.2.1 The results on the final report will be “delta9-THC content is above 1mg per dosage unit” or “delta9-THC content is below 1mg per dosage unit”.

11.1.2.2 The final report will not include any information regarding the dosage amount per container. The final report will include the quantity within the homogenous group and the results for the units analyzed within that homogenous group.

11.1.2.3 The state law definition of the term “THC” includes, but is not limited to, delta-7 THC, delta-8 THC, delta-9 THC and delta-10 THC. These additional cannabinoids are not currently included in the semi-quantitative evaluation of the product. If results are above 1mg of delta-9 THC per dosage unit, then these additional cannabinoids would be contributing to the established 1mg delta-9 THC content. If results are below 1mg of delta-9 THC per dosage unit and there are additional cannabinoids present, a note should be included on the report to

communicate to the submitting agency that the additional cannabinoids were evaluated qualitatively only.

13. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment will be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use. When casework samples are being processed/analyzed then brown paper (or other similar barrier) will be placed in between surfaces and specimens.

14. References

Gibson, C. *Drugs of Abuse*, 1997 ed., Drug Enforcement Administration, U.S. Department of Justice: Alexandria, VA.

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