Title: (CS-14 SOP) Analysis of Cannabis Plant Material

1. Introduction

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. Legislation has defined industrial hemp as being any Cannabis Sativa plant that has a total THC content of 0.3% or less based on dried vegetation. Alternatively, marijuana has been defined within legislation as dried Cannabis Sativa vegetation containing more than 0.3 % THC.

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. Besides vegetative material, the active THC chemical (as well as other cannabinoids) can be found within non-vegetative material such as food products (i.e., edibles) and concentrated extracts (e.g., hash/hashish/hash oil, vaping liquids, shatter, essential oils, and tinctures).

2. Scope

This procedure can be used for the analysis of submitted specimens for the determination of the amount of THC present. When evidence is received in the form of vegetative material then this procedure utilizes semi-quantitative measures in order to differentiate marijuana from hemp. 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). The analysis of non-vegetative materials for cannabinoids is outside the scope of this procedure.

3. Principle

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 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 in order to report marijuana as being within a submitted specimen.

4. Specimens

This procedure will only be used for the analysis of vegetative (or plant-like material (PM)) specimens in order 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.

Note: When vegetative material is analyzed, unless moisture is visible and able to be transferred to a paper-like material (e.g., brown paper, Kimwipe®, paper towel), samples will be considered to be in a dry state. If plant-like samples are found to not be in a dry state then the appropriate FSE2 and management will be consulted in order to come up with a resolution prior to analysis.

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 Digital camera

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

5.8 Vortex mixer

5.9 Automatic pipettes (with disposable tips)

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

5.11 Certified Reference Material (CRM) Solutions

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

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

6. Controls and Internal Standards

Positive/Negative Controls:
When applicable, store all materials according to their manufacturer's recommendations. If purchased, 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 Negative Control:

Any vegetative material that doesn't contain cannabinoid compounds may be used (e.g., tea, tobacco).

6.2 Positive Control:

6.2.1 Known marijuana vegetative material

6.2.2 Known hemp vegetative material

Preparation of solutions:

6.3 Internal Standard Solution (ISTD)
{Δ^9-THC-D_3 ; 0.1 mg/mL ; Total Volume = 10 mL}

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

6.3.2 This solution is stable (when stored in a freezer) for 1 year from its last verification or until the expiration date of the THC-D_3 CRM has been reached, whichever is earliest.

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

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

Can be prepared by:

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

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

6.4.3 Add 890 µL of methanol and mix.

6.4.4 Prepare fresh.

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. Procedure

7.1 Ensure the vegetative material is dry. If material is not dry consult a FSE2 or higher.

7.2 Perform a microscopic examination (e.g., using the MiScope®)

7.2.1 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.

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

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

7.3 Take a sample of the vegetative material (evidence and control samples). Homogenize and grind vegetative material with mortar and pestle.

7.4 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.

7.5 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.

7.6 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).

7.7 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.

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

Setting-up Instrument with Samples:

7.9 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.

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

7.10.1 Negative controls will be analyzed prior to evidentiary samples.

7.10.2 Blank samples (Methanol only – no internal standard) 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.

7.10.3 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.

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

7.12 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.

7.13 Sequence Verification:

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

7.13.2 Print the sequence list.

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

7.13.4 Once the autosampler vial check has been completed place an indication (e.g., 'sequence checked' or 'sequence verified') on the sequence page along with Analyst's initials and date.

7.14 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.

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

7.16 Begin the sequence and analyze the samples.

8. Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With documented approval from the Lead Examiner and appropriate management, the instrument conditions may be modified to adjust or improve the procedure. Documentation of such changes must be included with casework so that any instrumental parameter change can be associated with data and until the procedure has been updated. For more specific parameters, see method printout(s) contained within appropriate instrument binders or stored within the quality management system.

Gas Chromatograph/Mass Spectrometer (GC/MS):

Gas Chromatography			**Injector**	
Method Name:	Cannabis_10_1 split		Syringe Size	10 µL
Column Type	DB5-MS		Injection Volume	1 µL
Film Thickness	0.25 µm		Solvent A Wash	Ethyl Acetate
Column Length	30 m		Solvent A Volume	10 µL
Internal Diameter	0.25 mm		Pre-Inject (A Wash)	5 µL
Run Time	7.5 minutes		Post-Inject (A Wash)	5 µL
Initial Temperature	235 C		Solvent B Wash	Chloroform
Hold Time	0 minutes		Solvent B Volume	10 µL
Rate	30 C/min.		Pre-Inject (B Wash)	5 µL
Final Temperature	280 C		Post-Inject (B Wash)	5 µL
Hold Time	6 minutes		Sample Washes	0
Equilibration Time	0.5 min.		Sample Pumps	5
			Dwell Time (Pre-Inj.)	0 min.
Mass Spectrometer			Dwell Time (Post-Inj.)	0 min.
Polarity	Positive		L1 Air gap	0.2 µL
Tune File	ATUNE			
Ion Source	EI		**Inlet**	
Source Temperature	230 C		Mode	Split
Quad Temperature	150C		Inlet Temp.	250 C
Electron Energy	70eV		Gas Saver	On
Solvent Delay	2.5 min.		Split Ratio	10:1
Scan Time Segment			Split Flow	9 mL/min.
Time	2.5 min.			
Start Mass	40			
End Mass	550			
SIM Time Segment	**{Fentanyl}**			
Start Time	4.40 min.			
Resolution	Low			
Ion (m/z)	245			

9. 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 of the criteria below should be met in order to identify the appropriate analyte(s).

9.1 Microscopy:

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

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

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

9.1.4 Glandular hairs 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 hairs contain calcium carbonate.

9.2 Chromatography:

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics (e.g., reasonable peak shape, width, and resolution). In order 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.

9.2.1 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).

9.3 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.

9.4 Sequence Acceptance:

In order for a sequence to be acceptable:

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

9.4.2 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 in order to record events.

9.4.2.1 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).

9.4.3 Reporting Qualitative Results using Instrument Software

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

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

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

9.4.4 Reporting Qualitative Results using Manual Calculation

9.4.4.1 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% and the result will be reported as marijuana.

9.4.4.2 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% and the result will be reported as marijuana not being identified.

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

10. Calibration

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

11. Limitations

Cut-Off/Decision Point is 1% THC.

12. 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.

13. 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.