OSAC 2022-S-0014 Building an Analytical Scheme for the Assessment of Tetrahydrocannabinol (THC) in Suspected Marijuana Plant Material Samples

Seized Drugs Subcommittee
Chemistry: Seized Drugs/Toxicology Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

Draft OSAC Proposed Standard

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Prepared by
Seized Drugs Subcommittee
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There may be references in an OSAC Proposed Standard to other publications under development by OSAC. The information in the Proposed Standard, and underlying concepts and methodologies, may be used by the forensic-science community before the completion of such companion publications.

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To be placed on the OSAC Registry, certain types of standards first must be reviewed by a Scientific and Technical Review Panel (STRP). The STRP process is vital to OSAC's mission of generating and recognizing scientifically sound standards for producing and interpreting forensic science results. The STRP shall provide critical and knowledgeable reviews of draft standards or of proposed revisions of standards previously published by standards developing organizations (SDOs) to ensure that the published methods that practitioners employ are scientifically valid, and the resulting claims are trustworthy.

The STRP panel will consist of an independent and diverse panel, including subject matter experts, human factors scientists, quality assurance personnel, and legal experts, which will be tasked with evaluating the proposed standard based on a comprehensive list of science-based criteria.

For more information about this important process, please visit our website at: https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science/scientific-technical-review-panels.

Rationale:

The Farm Bill of 2018 removed hemp from the Controlled Substance Act Schedule I and defines it as "...the plant *Cannabis Sativa* L. and any part of the plant, including the seeds thereof, all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9-THC concentration of not more than 0.3% on a dry weight basis." As such, the OSAC Seized Drugs Subcommittee has drafted this standard to assist forensic science service providers to analyze seized drug evidence submitted to their laboratories as suspected Marijuana.

Standard Practice for

Building an Analytical Scheme for the Assessment of Tetrahydrocannabinol (THC) in Suspected Marijuana Plant Material Samples

1. Scope

1.1. This standard covers options for building an analytical scheme for the analysis and identification of suspected marijuana plant material in seized drugs.

1.2. This standard is intended for use by competent forensic science practitioners (FSPs) with the requisite formal education, discipline-specific training (see Practice E2917 and Practice E2326), and demonstrated proficiency to perform forensic casework.

1.3. This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2. Referenced Documents

2.1. ASTM Standards[2]

2.1.1. E1732 Terminology Relating to Forensic Science

2.1.2. E2326 Practice for Education and Training of Seized-Drug Analysts

2.1.3. E2548 Guide for Sampling Seized Drugs for Qualitative and Quantitative Analysis

2.1.4. E2549 Practice for Validation of Seized-Drugs Analytical Methods

2.1.5. E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs

2.2. Other Documents

2.2.1. Establishment of a Domestic Hemp Production Program; Federal Register, vol. 86, No, 11 January 19, 2021

2.2.2. SWGDRUG Recommendations Version 8.0, 2019 (https://www.swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%208_FINAL_ForPosting_092919.pdf)

2.2.3. United Nations Office on Drugs and Crime (UNODC) Recommended methods for the identification and analysis of *Cannabis* and *Cannabis* products, 2022.

(https://www.unodc.org/documents/scientific/Recommended_methods_for_the_identification_and_analysis_of_cannabis_and_cannabis_products.pdf)

3. Terminology

3.1. Definitions:

3.1.1. For definitions of terms used in this practice, refer to Terminology E1732.

3.2. Definitions of terms specific to this standard:

3.2.1. *Cannabis, n* - a genus of flowering plants in the family Cannabaceae of which *Cannabis sativa* is a species, and *Cannabis indica* and *Cannabis ruderalis* are subspecies thereof. *Cannabis* refers to any form of the plant where the total delta-9 tetrahydrocannabinol concentration on a dry weight basis has not yet been determined. (DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program)

3.2.1.1. *Discussion* - "The chemical and morphological distinctions by which *Cannabis* has been split into these subspecies are often not readily discernible, appear to be environmentally modifiable, and vary in a continuous fashion. For most purposes, it will suffice to apply the name *Cannabis* sativa to all *Cannabis* plants encountered." (United Nations Office on Drugs and Crime (UNODC) Recommended methods for the identification and analysis of *Cannabis* and *Cannabis* products, 2022.)

3.2.2. *decarboxylation, n* - the removal or elimination of a carboxyl group from a molecule or organic compound. (DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program)

3.2.3. *decision point, n* - an administratively defined cutoff or concentration that is at or above the method's limit of detection or limit of quantitation and is used to discriminate between positive and negative results. (Scientific Working Group for Forensic Toxicology (SWGTOX), "Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology." Journal of Analytical Toxicology, 37:7, 452-474, 2013.)

3.2.4. *dry weight basis, n* - a basis for expressing the percentage of a chemical in a substance after removing the moisture from the substance. Percentage of THC on a dry weight basis means the percentage of THC, by weight, in a *Cannabis* item, after excluding moisture from the item. (DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program)

3.2.5. *hemp, n* - the plant species *Cannabis sativa* L., and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a total delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis. (DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program)

3.2.6. *inconclusive results, n* - results that do not meet criteria for reporting, or were unsuitable due to analytical interferences or condition of the sample. (ANSI/ASB Standard 053)

3.2.7. *internal standard, n* - a compound of known concentration added to a sample to facilitate the qualitative identification and/or quantitative determination of the sample components (ISO 20752)

3.2.8. *marijuana, n* - or "marihuana" as defined in the Federal Controlled Substances Act (CSA) means all parts of the plant *Cannabis sativa* L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resin. The term "marihuana" does not include hemp and does not include the mature stalks of such plant, fiber produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt, derivative, mixture, or preparation of such mature stalks (except the resin extracted therefrom), fiber, oil, or cake, or the sterilized seed of such plant which is incapable of germination. "Marihuana" means all *Cannabis* that tests as having a THC concentration level of higher than 0.3 percent on a dry weight basis. (DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program)

3.2.9. *total THC, n* - the value determined after the process of decarboxylation, or the application of a conversion factor if the testing methodology does not include decarboxylation, that expresses the potential total delta-9 tetrahydrocannabinol content derived from the sum of the THC and THCA content and reported on a dry weight basis. (DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program)

3.2.9.1. *Discussion* - delta-9 THCA is a component of *Cannabis* that decarboxylates to delta-9 THC when heated. Also known as delta-9 Tetrahydrocannabinolic Acid or delta-9 THC Carboxylic Acid.

3.2.10. *THC, n* - for the purpose of this standard, refers primarily to delta-9 THC, but can include other THC isomers (e.g., delta-8-THC) depending on jurisdictional requirements.

3.2.11. *trichome, n* - hair-like projections from a plant epidermal cell. (United Nations Office on Drugs and Crime (UNODC) Recommended methods for the identification and analysis of *Cannabis* and *Cannabis* products, 2022.)

3.2.12. *isomers, n* - Compounds that have the same elemental formula, but have different structural configurations, and different physical and/or chemical properties. (Retrieved January 26, 2022 from OSAC lexicon, https://lexicon.forensicosac.org/)

4. Significance and Use

4.1. An analytical scheme is created to generate results for the assessment of THC in the analysis of suspected marijuana in seized-drug evidence. An analytical scheme is a combination of selected techniques used to reach a result, and is comprised of validated analytical methods that are appropriate for the analyte(s) or properties of interest. The combination of techniques chosen should aim to minimize false positives and false negatives.

NOTE 1 – This standard provides information that could assist in the differentiation between marijuana and hemp.

4.1.1. Identification only analytical schemes that do not include a decision point analysis or quantitative analysis cannot differentiate hemp from marijuana. They only provide information for the identification of *Cannabis.*

4.2. This Practice applies to plant material only, and does not cover derivatives, mixtures, or preparations such as concentrates, oils, or edibles.

4.3. These techniques cannot determine subspecies.

5. Sampling and Storage

5.1. If sampling in the field, follow DOA 7 CFR Part 990 Establishment of a Domestic Hemp Production Program.

5.2. Random sampling should be conducted (see Guide E2548) to address variations of THC content.

5.2.1. If one unit is received, sample portions from different areas within the unit.

5.2.2. If multiple units are received, do not combine. Use a sampling plan (e.g., hypergeometric approach, sample selection, sampling to penalty) to determine the number of units to sample individually.

5.2.3. Stems, stalks, and seeds should be excluded from sampling for qualitative and quantitative analysis.

5.3. Packaging/Storage - Fresh plant material should be packaged to allow the samples to dry (e.g., paper bags or perforated cardboard boxes), minimizing the amount of moisture and deterioration of the plant material.

5.3.1. THC is sensitive to air and UV light, therefore storage in a dark and cool place is recommended. (UNODC Recommended methods for the identification and analysis of *Cannabis* and *Cannabis* products, 2022).

5.3.2. The laboratory can establish additional procedures to refrigerate plant material samples.

5.3.3. If samples are received in a deteriorating state, the samples can still be analyzed. Document the state of the evidence in the case file.

6. Building an Analytical Scheme

6.1. The combination of analyses can be selected based on the information required (*i.e.* quantitation vs. decision point). Figure 1 illustrates the combination of testing that can be performed to identify marijuana. The individual tests are described in detail in the subsequent sections.

6.2. Minimum test requirements

6.2.1. Morphological Assessment

6.2.1.1. If a negative result is observed, this standard is no longer applicable.

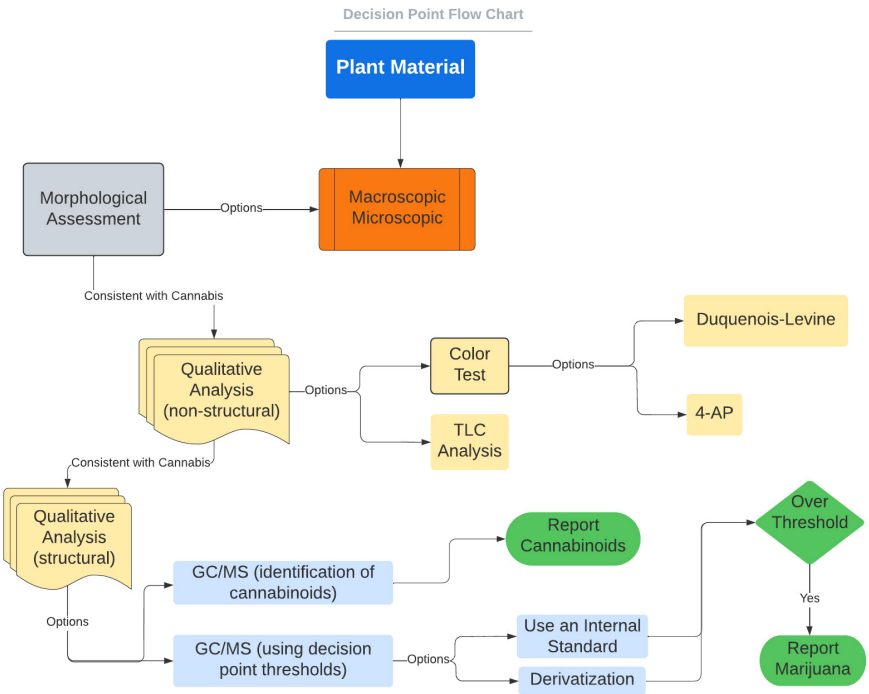
6.2.2. An analysis that provides structural data to confirm the presence of THC

6.2.2.1. This can be combined with the decision point analysis or full quantitation if those tests provide structural data.

6.2.3. An analysis to assess the amount of THC present in the item (decision point or full quantitation, or both).

6.3. The analytical scheme provides a scientifically supported conclusion when each technique achieves the level of selectivity required and the positive test results corroborate each other. (SWGDRUG Recommendations Version 8.0, SWGDRUG, 2019)

6.4. Additional testing can be completed as described in Section 8.



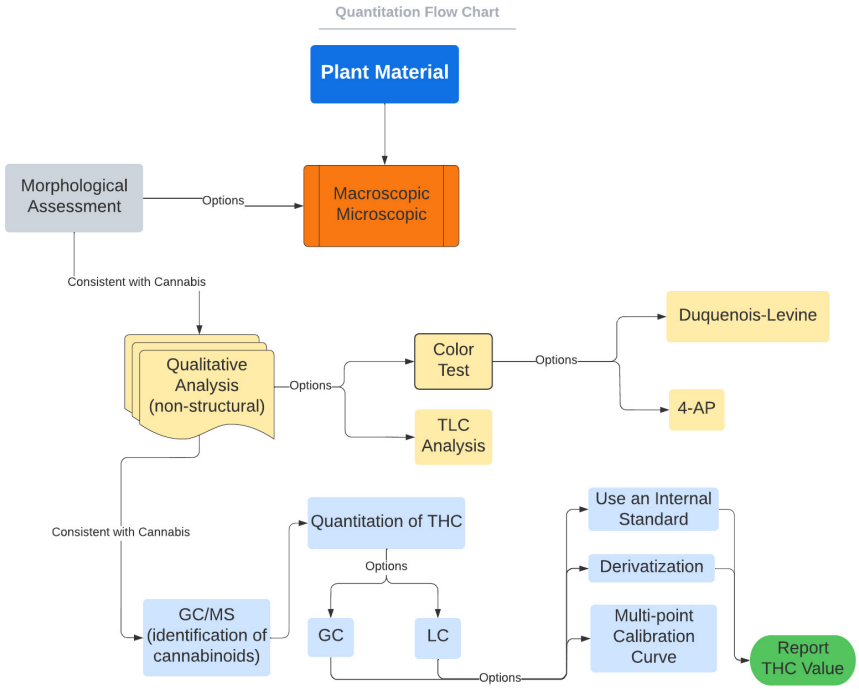


Figure 1: Suspected marijuana analysis scheme flowcharts

7. Morphological Assessment

7.1. Macroscopic Examination differentiates between plant material and non-plant material exhibits. Additional macroscopic observations can include documentation of color, stems/fruiting stalks, form (e.g., loose, compressed, ground), presence of seeds, palmate leaves with 3-11 leaflets, individual leaves with ellipsoid blade and serrated edges.

7.2. Microscopic Examination is conducted using a microscope with magnification of at least 10 times (e.g., 10-40x). Document observed features of the plant material; a picture can be captured to document the observations. These features can include:

7.2.1. Unicellular cystolithic trichomes found on the upper surface of the leaves with a characteristic bear-claw shape. See Figure 2 for an illustration.

7.2.1.1. Unicellular cystolithic trichomes contain a crystal of calcium carbonate at the base. Addition of dilute acid to the plant material surface, and observation of the resulting effervescence of the carbon dioxide formed as a result of the chemical reaction, can aid in distinguishing these hairs from other unicellular covering trichomes but is not required.

7.2.2. Multicellular glandular trichomes are found on the upper and lower surfaces of the leaves and have a shiny appearance. See Figure 2 for an illustration.

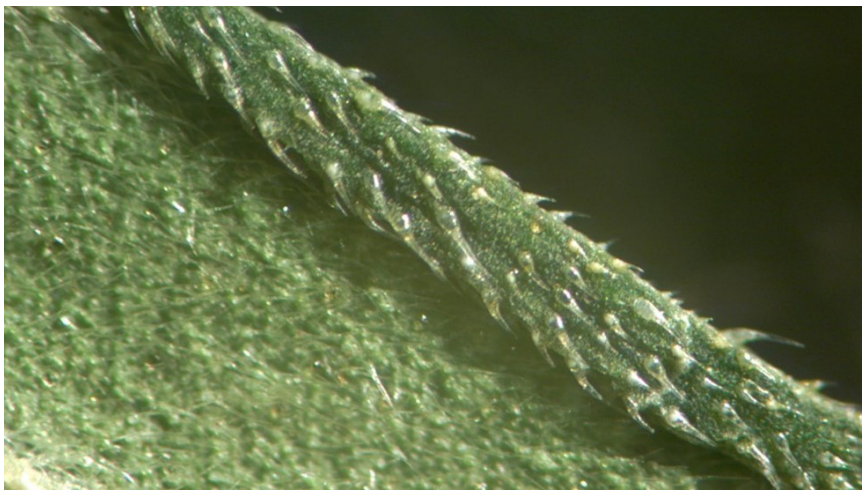


Figure 2: Figure 2 depicts cystolithic hairs and multicellular glandular trichomes of the *Cannabis* plant material at a magnification of 50X (source: DuPage county Sheriff's Office).

7.2.3. Observation of cystolithic hairs alone is not sufficient to report marijuana. The simultaneous presence of cystolithic trichomes on the upper surface of the leaves, along with the presence of non-cystolithic trichomes on the lower surface of the leaves must be observed (UNODC *Recommended methods for the identification and analysis of Cannabis and Cannabis products,* 2022).

7.2.4. It should be noted, however, that very immature seedlings and stems with no leaves attached cannot be definitively identified as *Cannabis* by botanical examination. (UNODC *Recommended methods for the identification and analysis of Cannabis and Cannabis products*, 2022).

8. Qualitative Analysis

8.1. Color Tests

8.1.1. Duquenois-Levine - Place a small amount of plant material (30 mg - 100 mg) in a test tube or other container. Cover with petroleum ether (or other organic solvent) to extract the cannabinoids into the solvent, filter or decant the solution to remove the residual plant material, evaporate to dryness, and add a small amount of Duquenois reagent and an equal amount of concentrated hydrochloric acid. A blue to purple color should develop within a few minutes. Add a small amount of chloroform or methylene chloride, shake, and let the layers separate. A violet to purple color in the organic layer indicates a positive test for cannabinoids.

8.1.1.1. Alternatively, the Duquenois reagent and concentrated hydrochloric acid can be added directly to a small amount of plant material.

8.1.1.2. Duquenois reagent consists of 0.5 mL acetaldehyde and 0.4 g vanillin in 20 mL of ethanol (e.g., 95%, 200 proof). Store the solution in a cool dark place and discard if it assumes a deep yellow color. (UNODC *Recommended methods for the identification and analysis of Cannabis and Cannabis products*, 2022) The reagent preparation can be scaled up or down as needed.

Figure 3A Figure 3B Figure 3C

Figures 3A-3C: Figure 3A depicts resulting observations when the Duquenois reagent is added to samples of cannabidiol (CBD), THC, and suspected marijuana plant material. Figure 3B depicts the resulting observations when an equal volume of concentrated hydrochloric acid is added. Figure 3C depicts the resulting observations when chloroform or methylene chloride is added. (source DEA)

8.1.2. 4-Aminophenol (4-AP) - A small amount of material (5 mg) can be placed in a test tube or spot plate and covered with the 4-AP Reagent A Solution. Add 2-4 drops of the Reagent B solution and wait 1-2 minutes. A blue color is indicative of the THC concentration being greater than the cannabidiol (CBD) concentration in the sample. A pink color is indicative of the THC concentration being less than the CBD concentration in the sample. If the concentration of THC is equivalent to the concentration of CBD, the test will be inconclusive.

8.1.2.1. Note: 4-AP Reagent A consists of 300 mg of 4-aminophenol, 5 mL of 2N HCl, and 995 mL of ethanol (e.g., 95%, 200 proof). Reagent B consists of 30 g sodium hydroxide, 300 mL of water, and 700 mL of ethanol. The reagent preparation can be scaled up or down as needed.

Figure 4A Figure 4B

Figures 4A and 4B: Figure 4A depicts resulting observations when the 4-AP test Reagent A added to samples of CBD, THC, and suspected marijuana plant material. Figure 4B depicts resulting observations when the 4-AP test Reagent B is added to the same samples of CBD, THC, and suspected marijuana plant material. The color blue will appear for a sample where THC was in greater concentration than CBD. (source: DEA)

8.2. Thin Layer Chromatography (TLC) - TLC can be used to compare the retention factor of the cannabinoid to that of a reference material. Possible TLC plates include silica gel G 250 micron. Possible solvent systems include 4:1 Petroleum Ether:Diethyl ether or 4:1

Hexane:Diethyl Ether. Visualization reagents that can be utilized are Fast Blue B, Fast Blue 2B spray and iodine (vapor).

8.2.1. Use method validation data to determine the acceptance criteria for retention factor comparisons. For example, the retention factor of the analyte can be within 5% of the retention factor of the reference material).

8.2.2. Alternatively, visually compare the sample spots to the reference spots at the greatest density in position and color. Some visualization reagents also allow for color differences between the substances present.

8.2.3. A picture of the TLC plate can be captured to document the observations.

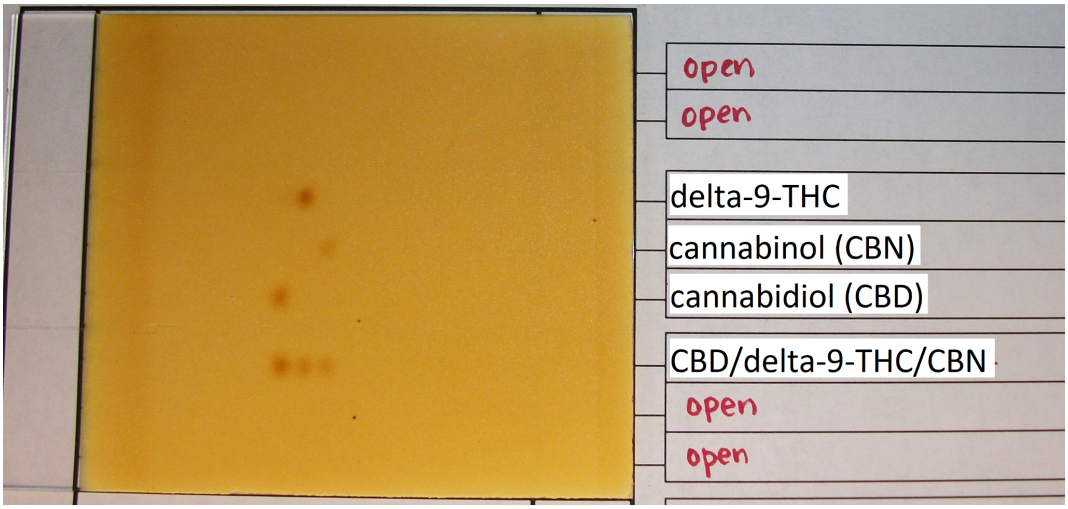


Figure 5: TLC plate developed using 4:1 petroleum ether:diethyl ether solvent system, silica gel G 250 micron plate, and visualized using iodine (vapor). Source: GBI DOFS

Figure 6: TLC plate developed using 4:1 hexane:diethyl ether solvent system and visualized using Fast Blue B spray. Source: NMS Labs

8.3. Gas Chromatography/Mass Spectrometry (GC/MS) - GCMS can be used for the identification of individual cannabinoids by comparing the retention time, mass spectrum, or both, to reference materials. 100% dimethylpolysiloxane, 5% (phenyl)-methylpolysiloxane, or 35% phenyl 65% dimethylpolysiloxane stationary phases are routinely utilized, with a 1µL injection volume and inlet temperature of at least 250 °C. The oven temperature program can vary. Individual method set points including inlet temperature, temperature program, flow rates, and column chemistry are evaluated during method development and tested during method validation.

8.3.1. Example oven temperature program: initial temperature 220°C, ramping at 1°C/minute, to a final temperature of 250°C, holding for 2 minutes.

9. Qualitative Analysis Using Decision Point Thresholds

9.1. The qualitative analysis of *Cannabis* samples using a decision point threshold can be performed in a number of ways. However, procedures typically follow the same basic steps.

NOTE 2 – Decision point thresholds are above those jurisdictionally defined as legal thresholds. The measurement uncertainty around the decision point should not encompass the legal threshold.

9.2. Sample Preparation

9.2.1. Drying (optional) - Dry plant material in an oven to obtain a sample dry enough to be homogenized (e.g., 1 hour at 60 °C).

9.2.2. Homogenization (optional) - Grind plant material with a device such as a disposable hand-held herb grinder or mortar and pestle. The material sampled for homogenization should exclude stalks, stems, roots, and seeds.

9.2.3. Extraction - Weigh a sample of plant material. The amount of sample to weigh is determined during validation of the method. If the material was not previously homogenized, crumble the weighed plant material (approximately 50 mg) and place into a test tube. Add extraction solution. Allow the plant material to extract for approximately 10-15 minutes or a period of time as determined during

method development or optimization. Vortex during this time. After extraction the solution can be filtered or centrifuged and the supernatant collected to remove particulates.

9.2.4. Use an Internal Standard – An internal standard solution can be added as part of the extraction solvent or added post extraction. Common internal standards utilized are 4-Androstene-3,17-dione, tribenzylamine (TBA), testosterone, and deuterated delta-9-THC. The concentration of the internal standard is typically the cutoff value for the defined decision point.

9.2.5. Aliquot Samples for Instrumental Analysis - Pipette a set volume of extract into a test tube and add internal standard if internal standard is not part of the extraction solvent. Vortex and transfer to an autosampler vial. If samples are believed to be high concentration, a dilution can be performed prior to analysis.

9.2.6. Derivatization (optional) - Derivatization can be conducted to obtain identification of THC and THCA separately as opposed to the total THC. Add the derivatizing agent (e.g., BSTFA-TMCS) to the extracted solution containing the internal standard or the dried residue from the extracted solution. The time and temperature at which the sample is derivatized as well as appropriate volumes of sample and derivatizing agent are determined during method validation.

9.3. Preparation of Calibrators and Controls

9.3.1. Decision Point with One-point Comparison

9.3.1.1. THC Calibrator at Decision Point - Prepare a THC standard by diluting a certified reference material (CRM) to an appropriate concentration in solvent. Then add a set volume of the standard to a test tube and add internal standard. Vortex and transfer to an autosampler vial.

9.3.1.2. CBD Conversion Control - Because of potential conversion of CBD to THC in the GC injection port when the sample is analyzed underivatized, procedures that use GC with no derivitzation should include a CBD conversion control. This can be prepared at a high concentration to demonstrate no conversion to THC or used to determine a cut-off concentration above which the THC result cannot be used. The FSSP should assess the conversion of CBD to THC during method development and validation. Frequency of injection port maintenance, consumables used in the injection port, and amount of CBD present in samples can affect the magnitude of conversion. This should be taken into account in validation experiments. Prepare a CBD standard by diluting a CRM to an appropriate concentration in solvent. Add a set volume of standard to a test tube and add internal standard. Vortex and transfer to an autosampler vial. The CBD conversion control should be analyzed throughout the run to monitor conversion.

9.3.1.3. THC Control at Decision Point - Prepare a second THC standard from a different CRM to an appropriate concentration in solvent. This can be done using a different lot of CRM, or a different manufacturer. Add a set volume of standard to a test tube and add internal standard. Vortex and transfer to an autosampler vial.

9.3.2. Decision Point with Internal Standard

9.3.2.1. THC control above decision point - prepare a THC standard by dissolving a reference material to an appropriate concentration (above

the decision point) in internal standard solution. Vortex and transfer to an autosampler vial.

9.3.2.2. THC control below decision point - prepare a second THC standard by dissolving a reference material to an appropriate concentration (below the decision point) in internal standard solution. Vortex and transfer to an autosampler vial.

9.3.2.3. Alternatively, controls can be prepared by extracting well-characterized plant reference materials that are above and below the decision point, using the same preparation procedure as sample(s) (see 8.2 above).

9.4. Instrumental Analysis

9.4.1. Gas Chromatography/Mass Spectrometry (GC/MS) - Acquire data by analyzing samples, calibrators, and controls (as applicable) on an appropriate, validated method. A 5% (phenyl)-methylpolysiloxane stationary phase is routinely utilized, along with a 1µL injection volume and an inlet temperature of 250 °C. The oven temperature program can vary. Data acquisition can be performed using full scan, SIM or SIM/scan. Individual method set points including inlet temperature, temperature program, flow rates, and column chemistry are evaluated during method development and tested during method validation.

9.4.2. Gas Chromatography/Flame Ionization Detection (GC/FID) - A second aliquot of the extracted sample is often analyzed by GC/FID. A 100% dimethylpolysiloxane stationary phase is often utilized. Instrument parameters are usually similar or the same as those used during the GC/MS analysis.

9.4.3. The method should be able to resolve delta-6a,10a-THC, delta-7-THC, delta-8-THC, delta-9-THC, delta-10-THC, and other THC isomers present in *Cannabis*. Method validation should include an assessment of interference to ensure the presence of multiple cannabinoids will not prevent the accurate determination of analytes of interest.

9.5. Data Analysis

9.5.1. If analysis is performed using a one-point threshold, create a one-point comparison and calculate the amount of THC in case samples and control samples.

9.5.2. If analysis is performed using a decision point with internal standard, calculate the ratio of sample THC to internal standard, using either peak area or peak height.

10. Quantitative Analysis

10.1. Drying

10.1.1. Quantitation is performed on plant material on a dry weight basis. During method validation, determine a drying time and temperature that renders the majority of samples seen in casework sufficiently dry for quantitative analysis. In order to dry the sample, one of the following procedures should be performed:

10.1.1.1. Moisture balance - The moisture balance will dry and weigh a sample. The loss on drying can be calculated and applied as a correction to the quantitative value obtained. The sample weighed on the moisture balance should not be the same sample used to perform the quantitation.

10.1.1.2. Obtain a weight of the sample. Dry plant material in an oven for approximately 1 hour at 60℃. Re-weigh the sample. If the change in

weight is greater than the acceptance criteria established in validation, place the sample back in the oven for further drying. Dry until the change in weight meets validation acceptance criteria.

10.1.1.3. Dry to constant weight - Continue drying until two consecutive weighings do not differ by more than 0.50 mg per g of substance taken. (USP-NF General Notices and Requirements, 6.40.20, GUID-6E790F63-0496-4C20-AF21-E7C283E3343E_6_en-US)

10.2. Homogenization

10.2.1. Grind plant material with a device, such as mortar and pestle. The material sampled for homogenization should exclude stalks, stems, roots, and seeds.

10.3. Weigh Quantitation Sample(s)

10.3.1. Weigh an aliquot of the homogenized material for extraction. The aliquot weight should be determined in method validation.

10.4. Extraction

10.4.1. Add extraction solution and internal standard solution (if applicable) and vortex/rotate . Sonicate/rotate the plant material to facilitate extraction of the cannabinoids. Sonicate/rotate the samples for approximately 10-15 minutes or a set period of time that is determined during validation of the method. Centrifuge/filter the samples and collect the supernatant to exclude particulates.

10.5. Dilution (Optional)

10.5.1. Pipette a set volume of supernatant into a test tube. Dilute with solvent or mobile phase at an appropriate volume so quantitated samples will fall within the calibration curve.

10.6. Additional Sample Preparation (Optional)

10.6.1. Perform a liquid/liquid extraction or solid phase extraction on a specified volume of supernatant. This step can be used for additional sample clean up prior to instrumental analysis if required.

10.7. Derivatization (Optional)

10.7.1. To prevent conversion of THCA to THC, the sample can be derivatized using BSTFA-TCMS. This will allow for quantitation of the components separately if analysis is performed on a platform such as GC/MS where decarboxylation will occur during analysis. The time and temperature at which the sample is derivatized as well as appropriate volumes of sample and derivatizing agent should be determined during method validation.

10.8. Preparation of Calibrators and Control

10.8.1. Prepare THC calibration standards by diluting a CRM to appropriate concentrations in the extraction/dilution solvent and internal standard (if applicable). Vortex/rotate and transfer to an autosampler vial.

10.8.2. Prepare THC control samples by diluting a different lot (or vendor) of CRM to appropriate concentrations in the extraction/dilution solvent and internal standard (if applicable). Vortex/rotate and transfer to an autosampler vial.

10.8.3. Prepare CBD conversion control if performing quantitative analysis using a heated method without derivatization by diluting a CRM to appropriate concentrations in the extraction/dilution solvent and internal standard (if applicable). Vortex/rotate and transfer to an autosampler vial.

10.8.4. Any calibrators and controls should be prepared using the same process as the samples. For instance, if sample preparation requires derivatization, the calibrators and controls should also be derivatized.

10.9. Instrumental Analysis

10.9.1. Analyze the THC calibration standards, THC controls, and samples on a validated method. The method should be able to resolve delta-6a,10a-THC, delta-7-THC, delta-8-THC, delta-9-THC, delta-10-THC, and other THC isomers present in *Cannabis*. Method validation should include an assessment of interference to ensure the presence of multiple cannabinoids will not prevent the accurate quantitation of analytes of interest.

10.9.2. Multi-point calibration curves are preferable to calculate the amount of THC in case samples.

10.9.2.1. The recommended minimum is three calibrators, not including the origin.

10.9.2.2. A single-point calibration can be valid for quantitation as long as method validation includes assessing linearity, and the y-intercept is shown to be negligible.

10.9.3. Establish acceptance criteria for any control samples analyzed. These criteria must be met for the data to be considered acceptable and results reported for case samples

10.9.4. Instrumental analysis can be performed on a variety of platforms. Example instrument parameters are listed below. Alternatives are acceptable as long as the method is validated.

10.9.4.1. LC:

- C18 columns are routinely used.
- Routine mobile phases can include 0.1% v/v formic acid in water:0.05% v/v formic acid in methanol or 0.1% TFA in water:0.1% TFA in acetonitrile.

10.9.4.2. GC

- Routine stationary phases can include 100% dimethylpolysiloxane, 5% (phenyl)-methylpolysiloxane, or 35% phenyl 65% dimethylpolysiloxane.

11. Uncertainty of Measurement

11.1. Calculate the uncertainty of measurement for quantitative analysis and qualitative decision point analyses at the threshold value.

11.1.1. Measurement uncertainty is reported with quantitative results and for statements of conformity (see ISO/IEC 17025).

11.1.2. The uncertainty for the method should be calculated at the decision point threshold. As a quantitative value is not reported, a specific uncertainty value is not reported for samples.

11.2. There are a variety of approaches that can be used for the determination of measurement uncertainty. At a minimum, uncertainty from sampling and the method of analysis should be included when determining uncertainty of measurement for quantitative analysis (Supplemental Document SD-4: Measurement Uncertainty for Quantitative Determinations in Seized Drug Analysis, SWGDRUG, 2013). The following are examples of components that can be assessed when calculating measurement uncertainty:

11.2.1. Calibration material

11.2.2. Balances used to weigh aliquots

11.2.3. Uncertainty associated with volumetric glassware and pipettes

11.2.4. Measurement reproducibility/imprecision

11.2.5. Method bias

12. Reporting Language

12.1. THC is not identified in analysis

12.1.1. Samples can be reported as THC not present/detected or marijuana negative/not detected

12.2. Qualitative Analysis Using Decision Point Threshold

12.2.1. Above Decision Point Threshold

12.2.1.1. If the testing scheme includes morphological examination to identify the *Cannabis* plant and the amount of THC or total THC is greater than the decision point, the sample can be reported as "marijuana" or the term defined in the respective State law.

12.2.1.2. The THC content can be reported as greater than the decision point threshold.

12.2.2. Below Decision Point Threshold

12.2.2.1. If the testing scheme includes an analysis to identify the *Cannabis* plant and the amount of THC or total THC is less than the decision point, the sample can be reported as "inconclusive for marijuana" or the THC content as less than the decision point threshold.

12.2.3. Alternatively reports can contain a statement with an explanation of the results if greater than/less than the decision point is not indicated for each item. See examples below:

12.2.3.1. This item was tested using Gas Chromatography-Mass Spectrometry (GC-MS), macroscopic examination, microscopic examination, and color test(s). The <FSSP Name> uses a decision point threshold of <## %> delta-9-tetrahydrocannabinol (THC) content in plant material, without decarboxylation of tetrahydrocannabinolic acid (THCA), to conclusively identify marijuana. Items above <decision point threshold value> are reported as "marijuana" and items below <decision point threshold value> are reported as "Inconclusive, not able to differentiate between marijuana or hemp." Quantitative (purity) analysis was not performed.

12.2.3.2. Inconclusive - A determination of inconclusive indicates that the plant material was unable to be identified as marijuana or hemp based on the analytical results obtained from the analytical scheme.

12.3. Quantitative Analysis

12.3.1. Purity values with associated uncertainty are reported when quantitative analysis is performed on samples. The report can contain a result pertaining to "marijuana" or the term defined in the respective State law as appropriate.

12.3.1.1. Quantitative result is above 0.3% THC: Marijuana can be reported in addition to the purity.

12.3.2. Quantitative result is below 0.3% THC or the uncertainty of measurement encompasses 0.3% THC: Samples can be reported as *Cannabis*, hemp, or marijuana negative/not detected along with the purity.

12.3.3. Quantitative result for THC is below the limit of quantitation (LOQ): In this situation a purity value is not reported. Samples can be reported as not present above the reporting limit, below the value of the low calibrator (##), or below the LOQ (##). In regards to the marijuana result, samples can be reported as *Cannabis*, hemp, or marijuana negative/not detected.

13. Quality Assurance

13.1. Quality control samples will be analyzed with each instrumental analytical run. Establish acceptance criteria for control samples. These criteria must be met for the results obtained for unknown samples to be reported.

13.1.1. Negative controls:

13.1.1.1. Solvent blanks are used to determine that the instrument is free from contamination/carryover.

13.1.1.2. Method or procedural blanks (e.g., internal standard blank)/reagent blanks are quality control samples used to assess the process. They ensure that the reagents used to prepare the samples are free from contamination.

13.1.2. Positive controls:

13.1.2.1. Positive controls are samples of known concentration analyzed on the same method as casework samples. They ensure the method is producing acceptable results. Positive control concentrations are chosen so they encompass the analytical measurement range. In analyses where there is a legal threshold, controls can be prepared above and below the legal limit.

13.2. Validation

13.2.1. Method validations should be to conducted to evaluate each method for the following when applicable (see Practice E2549): sensitivity, specificity, selectivity, detection limits, accuracy, precision, effects of decarboxylation, and any interferences from other cannabinoids (e.g., in situ production) or other commonly seen substances.

13.3. Limitations

13.3.1. Limitations associated with instrumental analysis

13.3.1.1. Cannabinoid acids decarboxylate in a GC injection port if samples are not derivatized. If analysis is performed by GC/MS or GC/FID without derivatization, the delta-9-THC result will include free delta-9-THC and decarboxylated THCA. CBDA will also decarboxylate to CBD.

13.3.1.2. It is possible for cannabinoids to interconvert to some extent under different conditions. The potential for degradation and conversion should be evaluated during method development and validation and monitored when necessary during analysis of casework.

13.3.2. Limitations associated with color tests

13.3.2.1. Color tests are presumptive tests. Other cannabinoids and non-cannabinoid compounds with similar structural features can result in the same color changes as the analyte of interest.

13.3.3. Limitations associated with TLC

13.3.3.1. Thin layer chromatography is a comparison technique. More than one compound can have the same retention factor. Potential interferences should be assessed and documented during validation.

14. Keywords

14.1. *Cannabis*; Marijuana; Tetrahydrocannabinol; Seized Drugs

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599 Appendices

600 XI. Table of Summary of Analytical Tests

Technique	Qualitative	Decision Point Threshold	Quantitative
Morphological Assessment	X		
Duquenois Levine	X		
FBBB	X		
4-AP	X		
TLC	X		
GC/FID	X	X	X
GC/MS	X	X	X
LC-UV	X	X	X
LC/MS	X	X	X

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603 XII. Examples of Analytical Schemes

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605 Example Scenario 1: Determine if the sample is Marijuana using a full mass spectral scan decision point
606 threshold.

Technique	Result	Test Type
Morphological Assessment	cystolithic hairs	Qualitative
GC/MS	THC over the decision point threshold	Decision Point Threshold

607 Reporting: The sample is Marijuana.

608

609 Example Scenario 2: Plant material submitted to determine if the sample is Marijuana using a decision
610 point threshold.

Technique	Result	Test Type
Morphological Assessment	cystolithic hairs	Qualitative
GC/MS	Delta-9-THC	Qualitative
GC/FID	THC over the decision point threshold	Decision Point Threshold

Reporting: The sample is Marijuana.

Example Scenario 3: Plant material with THC content more than CBD. Determine if the sample is Marijuana using a decision point threshold.

Technique	Result	Test Type
Morphological Assessment	cystolithic hairs	Qualitative
4-AP	Blue	Qualitative
GC/MS	THC over the decision point threshold	Decision Point Threshold

Reporting: The sample is Marijuana.

Example Scenario 4: Plant material with THC less than the decision point and delta-8-THC is present. Determine if the sample is Marijuana using a decision point threshold.

Technique	Result	Test Type
Morphological Assessment	cystolithic hairs	Qualitative
GC/MS (full scan)	Delta-9-THC below the decision point threshold and contains delta-8-THC	Decision Point Threshold

Reporting: The sample is inconclusive for Marijuana and contains delta-8-THC.

Example Scenario 5: Plant material with THC content less than CBD, but contains high concentrations of CBN (a known false positive on the 4-AP test). Determine if the sample is Marijuana using a decision point threshold.

Technique	Result	Test Type

Morphological Assessment	cystolithic hairs	Qualitative
4-AP	Blue (false positive)	Qualitative
GC/MS (full scan)	CBD > THC and THC over the decision point threshold. High CBN observed.	Decision Point Threshold

Reporting: The results are inconclusive.