TENNESSEE HEMP PLAN
Submitted under
7 C.F.R. § 990
Domestic Hemp Production Program

Tennessee's plan for monitoring and regulating the production of hemp complies with the requirements of 7 C.F.R. § 990. The hemp regulatory program, in place since 2015, is a combination of statutes, rules, protocols, and SOP's. The Tennessee General Assembly passed Public Chapter 916 in 2014 with amendments in 2019 by Public Chapter 87(*Attachment A*) that authorized the department of agriculture to promulgate rules to implement the program. The rules were first issued in January 2015 and rewritten in September 2021(*Attachment B*). The statute and rules in their current form provide the framework of the state hemp program plan submitted under 7 C.F.R. § 990.3, 990.6, and 990.7.

1. A Plan to maintain relevant producer and land information under 990.3(a)(1)]

1.1 The information required under rule 0080-06-28-.03 [Attachment B] shall be maintained in the records of the department of agriculture.

1.2 The following information shall be reported to USDA in the required format by the first day of each month:

1.2.1 The information collected under 0080-06-28-.03(2).

1.2.2 Any other information required by 990.70(a)(1).

1.2.3 Any change in the status of a licensee.

2. A Plan for accurate and effective sampling and testing using post-decarboxylation or similarly reliable methods under 990.3(a)(2).

2.1 The department's laboratory will use liquid chromatography tandem mass spectrometry (LC-MS/MS). This is a "gentler," lower temperature technique than methods involving gas chromatography, meaning that we can look for cannabinoids that have not yet undergone complete decarboxylation. However, we can produce equivalent results to methods that quantitate cannabinoids post-decarboxylation. This is accomplished by mathematically combining the THC mass contributions of Δ9-THC and THCA (on a dry weight basis). The generally accepted formula for doing so is: $Conc\ Total\ THC$=($Conc\ THCA$∗0.877)+$Conc\ \Delta 9\text{-}THC$. This equation is derived from the molecular weights of Δ9-THC and THCA to compensate for the mass lost during the decarboxylation of THCA to Δ9-THC.

2.2 See also Attachment A, § 43-27-105**,** Attachment B, 0080-06-28-.06, Attachment C for testing protocols, and Attachment E for sampling procedures.

2.3 Test results will be sent to USDA to determine compliance.

3. Procedures to do performance-based sampling under 990.3(a)(2)(iii)

3.1 See Attachment D for performance-based sampling.

4. Plan for disposal and remediation procedures under 990.3(a)(6).

4.1 Remediation and Disposal Guidelines for Hemp Growing Facilities, U. S. Domestic Hemp Production Program, Issued January 15,2021, is adopted by reference as if fully stated herein as a part of this plan.

4.2 See also Attachment A, § 43-27-106 and Attachment B, 0080-06-28-.06.

5. Plan for conducting annual inspections of a random sample of licensed producers under 990.3(a)(7).

See Attachment A, § 43-27-103 and Attachment B, 0080-06-28-.06.

6. Plan for collection and submission of information under 990.70.

6.1 The following information shall be reported to USDA and FSA in the required format by the first day of each month:

1.2.1 The information collected under 0080-06-28-.03(2).

1.2.2 Any other information required by 990.70(a)(1).

1.2.3 Any change in the status of a licensee.

6.2 See also Attachment B, 0080-06-28-.04.

6.3 Total acreage of hemp planted, harvested, and disposed of or remediated will be included in the monthly reports.

7. Plan to comply with enforcement procedures under 990.6.

7.1 See Attachment A, § 43-27-103, through 107 and Attachment B, 0080-06-28-.06.

7.2 Producers will not receive more than one negligent violation per growing season.

7.3 Producers shall report the results of their remediation plan to the department for two years from the date of the violation.

8. Certification that the state has resources and personnel to carry out required Farm Bill practices and procedures 990.3(a)(9).

See Attachment F.

ATTACHMENT A

Statutory Authority

HEMP

43-27-101. Chapter definitions.

As used in this chapter:

(1) Commissioner means the commissioner of agriculture;

(2) Department means the department of agriculture;

(3) Hemp means the plant 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 delta-9 tetrahydrocannabinol (THC) concentration of not more than three-tenths of one percent (0.3%) on a dry weight basis; and

(4) THC means delta-9 tetrahydrocannabinol.

43-27-102. Hemp license Requirements Records.

(a) Any person who produces hemp in this state shall obtain an annual license from the department.

(b) In order to obtain and maintain a hemp license, a person must:

(1) Submit to the department a description of all land on which the person produces hemp in this state, to include global positioning system coordinates and other information sufficient to identify the property;

(2) Submit to the department any other information prescribed by rules as necessary for the efficient enforcement of this chapter;

(3) Consent to reasonable inspection and sampling by the department of the person's hemp crop and inventory; and

(4) Not be convicted of a state or federal felony drug offense within the previous ten (10) years.

(c) The department shall maintain all records that the department creates, or that are submitted to the department, for regulation of hemp in this state for a period of at least five (5) years.

43-27-103. Prohibited acts.

The following acts within this state are prohibited:

(1) Possession of rooted hemp by any person, other than a common carrier, without a valid license issued by the department;

(2) Possession of cannabis with THC concentrations greater than three-tenths of one percent (0.3%) on a dry weight basis;

(3) Failure to pay upon reasonable notice any license, sampling, or inspection fee assessed by the department;

(4) Violation of this chapter or any rule promulgated under this chapter; or

(5) Willful hindrance of the commissioner or the commissioner's authorized agent in performance of their official duties.

43-27-104. Authority of commissioner.

(a) The commissioner is authorized to:

(1) Administer this chapter;

(2) Take all action necessary to obtain primary regulatory authority over the production of hemp in this state, as authorized by Section 297 of the Agriculture Improvement Act of 2018 (Public Law 115-334);

(3) Promulgate rules in accordance with the Uniform Administrative Procedures Act, compiled in title 4, chapter 5, as necessary for regulation of hemp in accordance with the federal Agriculture Improvement Act of 2018 and as determined by the commissioner to be necessary for the efficient enforcement of this chapter;

(4) Determine requirements for and issue licenses for the production of hemp in this state;

(5) Deny or revoke licenses and issue civil penalties up to one thousand dollars ($1,000) for each violation of this chapter or its rules;

(6) Establish reasonable fees for hemp licenses necessary to implement and administer a hemp program in this state on an ongoing basis. All revenue collected from fees established under this subdivision (a)(6) must be used exclusively for administration of a hemp regulatory program by the department;

(7) Require the maintenance or filing of records; and

(**8)** Enter during normal business hours any premises or conveyance of a person licensed under this chapter for purposes of inspection, sampling, and observation and copying of records required under this chapter.

(b) All rules promulgated by the department prior to July 1, 2019, for regulation of industrial hemp are null and void immediately upon rules promulgated to effectuate chapter 87 of the Public Acts of 2019 taking effect. Within one hundred twenty (120) days of chapter 87 of the Public Acts of 2019 becoming law, the department shall promulgate rules necessary to

effectuate the purposes of this chapter. The commissioner is authorized to file emergency rules under 4-5-208 as necessary for compliance with this subsection (b).

43-27-105. Enforcement of chapter Sample and analysis of hemp produced in state.

(a) The department shall enforce this chapter in a manner that may reasonably be expected to prevent production or distribution of cannabis with THC concentrations exceeding three-tenths of one percent (0.3%) on a dry weight basis, including random inspections and sampling of hemp licensees to ensure compliance with this chapter and rules promulgated under this chapter.

(b) The department shall sample and analyze hemp produced in this state and hemp products distributed in this state for THC concentrations, tested according to protocols prescribed by rule under this chapter. Departmental testing methods shall employ liquid chromatography tandem mass spectrometry, in a manner similarly reliable to post-decarboxylation, to determine a cannabinoid profile of samples tested, including their THC concentrations.

43-27-106. Stop movement or destruction order for plant or product exceeding authorized concentrations Penalties Evidence.

(a) When the commissioner or the commissioner's authorized agent finds any cannabis or cannabis product to contain THC concentrations greater than three-tenths of one percent (0.3%) on a dry weight basis, the commissioner may issue either a written stop movement order or written destruction order for the plant or product, as appropriate to best serve the public interest and purpose of this chapter.

(b) Any person who negligently violates this chapter or rules promulgated under this chapter is subject to administrative action by the department including denial or revocation of any license issued under this chapter; issuance of stop movement orders, destruction orders, and civil penalties; and actions for injunction. Negligent violations of this chapter or rules promulgated under this chapter shall not be the basis for criminal prosecution of any person.

(c) Any person who violates this chapter or rules promulgated under this chapter with a culpable mental state greater than negligence shall be subject to prosecution under any applicable state or federal law. If the department determines that a person has violated this chapter or rules promulgated under this chapter with a culpable mental state greater than negligence, the department shall report the matter to the Tennessee bureau of investigation and the United States attorney general.

(d) In all proceedings brought to enforce this chapter, proof of testing consistent with rules promulgated under this chapter showing THC concentrations greater than three-tenths of one percent (0.3%), but not greater than one percent (1.0%), on a dry weight basis is prima facie evidence of a negligent violation of this chapter.

(e) In all proceedings brought to enforce this chapter, the following are prima facie evidence of violation with a culpable mental state greater than negligence:

(1) Proof of testing consistent with rules promulgated under this chapter showing THC concentrations greater than one percent (1.0%) on a dry weight basis;

(2) Three (3) violations within a five-year period for possession of rooted hemp without a valid license issued by the department; or

(3) Violation of any stop movement or destruction order issued under this chapter.

(f) Any person whose license is revoked for violation of this chapter or rules promulgated under this chapter is ineligible for reissuance of the license for a period of at least five (5) years.

43-27-107. Jurisdiction for violations.

When the commissioner has reason to believe that a person is causing or has caused a violation of this chapter or the rules promulgated under this chapter, the commissioner may initiate proceedings in either the chancery court of Davidson County or the chancery court of the county where the violation occurred, for injunctive relief to prevent the continuance of the violation or to correct the conditions resulting in the violation.

43-27-108. Exemption from other applicable statutes and rules not provided by this chapter.

This chapter does not exempt any person from enforcement of statutes and rules applicable to particular uses of hemp, including, but not limited to, food safety statutes and rules for distribution of food products; feed statutes and rules for distribution of commercial feed; and seed statutes and rules for distribution of seed.

ATTACHMENT B

Chapter 0080-06-28
Hemp

0080-06-28-.01 Scope.

This chapter applies to any person who:

(a) grows rooted hemp;

(b) possesses rooted hemp; or

(c) possesses harvested hemp for transportation to anyone who treats or transforms it for distribution in commerce.

Authority: T.C.A. §§ 4-3-203 and 43-7-104.

0080-06-28-.02 Definitions.

(1) Terms in this chapter share those meanings of terms in T.C.A. § 43-27-101 et seq.

(2) When used in this chapter, unless the context requires otherwise:

(a) Act means T.C.A. § 43-27-101 et seq.;

(b) Cannabis plant means any plant or any part of a plant of the genera Cannabis and includes hemp;

(c) Culpable mental state greater than negligence means to act intentionally, knowingly, willfully, or recklessly;

(d) Disposal means an activity that transitions non-compliant hemp into a non-retrievable or non-ingestible form. Such activities include, but are not limited to plowing, tilling, or disking plant material into the soil; mulching, composting, chopping or bush mowing plant material into green manure; or burying plant material into the earth and covering with soil;

(e) Grow means to cultivate plants with attached roots;

(f) Growing area means any contiguous land area licensed for the growth of hemp. Bifurcation of a growing area by roads, fencing, or the like shall not render the area non-contiguous under this definition;

(e) Hemp producer means a person that produces hemp for harvest;

(f) Hemp broker means a person that purchases and sells hemp plants grown by others;

(g) Hemp propagator means a person that produces clones or seedlings for retail sale;

(h) Key participant means a sole proprietor, a partner in a partnership, or a person with

executive managerial control in a corporation such as, a chief executive officer, chief operating officer, or chief financial officer;

(i) Lot means a contiguous area in a field, greenhouse, or indoor growing structure containing the same variety or strain of cannabis plant throughout the area;

(j) Move, distribute, transport, or similar words mean to relocate in any manner an item from one real property to another;

(k) Negligence means failure to exercise the level of care that a reasonably prudent person would exercise in complying with the act or this chapter;

(l) Person means an individual, partnership, corporation, or any other form of legal entity;

(m) Remediation means the process of rendering non-compliant cannabis, compliant by removing and destroying flower material, while retaining stalk, stems, leaf material, and seeds. Remediation can occur by shredding the entire plant into a biomass like material, then re-testing the shredded biomass material for compliance;

(n) Sample means to take material, or the material taken from a location licensed by the department;

(o) Stop movement order means a written directive issued by the department to prohibit or limit the movement of plants or plant parts;

(p) THC means total delta-9 tetrahydrocannabinol; and,

(q) USDA means United States Department of Agriculture.

Authority: T.C.A. §§ 4-3-203 and 43-27-104.

0080-06-28-.03 License Application and Fees.

(1) A hemp license is required to possess rooted hemp and is issued to each person for each physical address where the person grows or possesses rooted hemp.

(2) Application for a license shall be made on forms provided by the department, which shall be completed in full and shall include:

(a) Name of applicant;

(b) Date of birth of any key participant;

(c) Proof of one of the following for any applicant that is not an individual or a general partnership:

1. Applicant’s registration in its state of incorporation; or,

2. Applicant’s business license issued by a local governmental authority;

(d) Contact information for applicant, to include name of key participants, telephone number, email address, and address of the principal place of business;

(e) Address of the location to be licensed for growth of hemp and description of all growing areas at the location, including total number of growing acres and Global Positioning System (GPS) coordinates from the areas’ central most points; and

(f) Criminal history report for key participants in the form of the Federal Bureau of Investigation's Identity History Summary.

(g) Other information as required by the department.

(3) Licensees shall notify the department of any changes to contact information of an application within 30 days after the change takes place.

(4) The department will not refund fees for early termination of any license issued under this chapter.

(5) Licenses issued under this chapter are not transferable from person to person or location to location.

(6) The annual fee for a hemp producer license is assessed under T.C.A. §43-1-703(f) and is determined according to the total size of growing area(s) at the licensed address:

(a) Less than 5 acres: Tier 6 fee;

(b) 5 acres to 20 acres: Tier 7 fee;

(c) More than 20 acres: Tier 8 fee.

(7) The annual fee for a hemp broker license is assessed under T.C.A. §43-1-703(f) and is a Tier 7 fee.

(8) The annual fee for a hemp propagator license is assessed under T.C.A. §43-1-703(f) and is a Tier 7 fee.

(9) The annual fee for any combination of a hemp producer, broker, or propagator license is a single fee equal to the highest amount of the license for which the applicant requests.

(10) License applicants shall submit an application and license fee to the department on or before July 1 of each year. The annual license fee shall be waived for any institute of higher education that offers programs of study in agricultural sciences seeking licensure for a growing area on university property. Licenses expire on June 30 following their issuance.

(9) The department may deny any application for licensure that is not completed in accordance with this rule.

(10) Any person who materially falsifies any information in their application shall be ineligible to participate in the program.

(11) A person convicted of any drug-related felony offense in any state or federal jurisdiction within the previous ten (10) years shall not participate in the program or grow hemp for ten (10) years from the date of the conviction. An exception applies to a person who was lawfully growing hemp under section 7606 of the Agricultural Act of 2014 (7 U.S.C. 5940) before December 20, 2018, and whose conviction also occurred before that date.

Authority: T.C.A. §§ 4-3-203 and 43-27-104.

0080-06-28-.04 USDA Reports.

All producers shall report hemp crop acreage to the USDA Farm Service Agency within 30 days of obtaining a license and shall provide the following information:

(a) Street address and the GPS location of the lot, greenhouse, building, or site hemp will be grown.

(b) Acreage dedicated to the production of hemp, or greenhouse or indoor square footage dedicated to the production of hemp.

(c) The hemp license number.

Authority: T.C.A. §§ 4-3-203 and 43-27-104.

0080-06-28-.05 Movement Permits.

(1) Licensees shall not move rooted hemp plants without a valid movement permit issued by the department. Licensees shall not move any hemp to anyone who treats or transforms harvested hemp for distribution in commerce without a valid movement permit issued by the department.

(2) Hemp movement permits are required per vehicle per day. To receive a movement permit, the licensee shall submit a movement permit request on forms provided by the department, which may require:

(a) The hemp license number of the person requesting the permit;

(b) Origin and destination of movement;

(c) Date of intended movement;

(d) Weight, volume, or number of units of material to be moved.

(3) The department may deny any application for a movement permit that is not completed in accordance with this rule.

Authority: T.C.A. §§ 4-3-203 and 43-27-104.

0080-06-28-.06 Sampling and Inspections.

(1) The department or a designated representative may enter during normal business hours any location, licensed by the department, for purposes of inspecting any cannabis plant, record, or other material as necessary for the efficient enforcement of the Act and this chapter.

(2) Sampling.

(a) The department or a designated representative, trained and approved by the department, may conduct sampling of any cannabis plant or other material at a location licensed by the department.

(b) A sample collected according to uniform protocols approved by the commissioner shall be deemed representative of the location, growing area, or lot from which the sample was obtained.

(c) Within 30 days prior to the anticipated harvest the department or a designated representative shall collect samples from the flower material for THC level testing.

(d) During a scheduled sample collection, the producer or an authorized representative shall be present at the growing site.

(e) A producer shall not harvest the hemp crop prior to samples being taken.

(f) Samples from different lots shall not be comingled.

(3) The protocols employed by the Tennessee Department of Agriculture define the preparation and analysis of hemp samples for the quantitative determination of cannabinoids, including delta-9-THC, on a dry weight basis and is conducted in a manner similarly reliable to post-decarboxylation.

(4) The department and growers may utilize private laboratories for testing official samples, if the laboratory meets the standards set by USDA for such work and the sample is sent directly to the laboratory by the sampler. The department's laboratory shall serve as the reference laboratory for all samples.

(5) Any sample test result higher than 0.3% THC concentration on a dry mass basis shall be conclusive evidence that one or more cannabis plants from the area sampled contains a THC concentration in excess of that allowed under the Act and shall be grounds for destruction or remediation.

(6) Licensees shall pay a Tier 4 laboratory analysis fee under T.C.A. §43-1-703(f) for each sample tested by the department.

Authority: T.C.A. §§ 4-3-203 and 43-27-104.

0080-06-28-.07 Violations.

(1) Violations shall include, but not limited to:

(a) Failure to provide a legal description of land on which the producer produces hemp;

(b) Failure to obtain a license;

(c) Production of cannabis at a THC concentration exceeding the acceptable limits;

(d) Possess or grow rooted hemp outside a licensed growing area unless it is under immediate transport to a licensed growing area;

(e) Failure to provide full and accurate information regarding the person's acquisition, cultivation, and distribution of hemp when requested by the department;

(f) Cultivate, move, or distribute cannabis plants other than hemp;

(g) Interfere with an authorized representative of the department in the performance of his duties;

(h) Market or represent hemp or hemp products to be marijuana or any illicit substance in any form;

(i) Failure to destroy or remediate non-compliant hemp;

(j) Failure to comply with a stop movement order;

(k) Violate any state or federal quarantine or order issued by the department.

(2) All violations are committed with negligence or with a culpable mental state greater than negligence.

(3) All negligent violations shall be corrected using a corrective action plan and shall, at a minimum, include the following items:

(a) A reasonable date by which the producer shall correct the negligent act;

(b) That the producer will periodically report to the department progress in correcting the violation; and

(c) The department shall conduct an inspection to determine if the action plan has been implemented.

(4) Hemp producers do not commit a negligent violation under this paragraph if they make reasonable efforts to grow compliant hemp and it does not have a THC concentration of more than 1% on a dry weight basis.

(5) A producer that negligently violates the act or this chapter three (3) times within a five (5) year period shall be ineligible to produce hemp for five (5) years beginning on the date of the third violation.

(6) Culpable violations shall be reported to the U. S. Attorney General and the chief law enforcement of the state.

(6) A person is responsible for violations of the Act or this chapter when committed by either the person or his agent.

Authority: T.C.A. §§ 4-3-203 and 43-27-104.

ATTACHMENT C

TECHNICAL PROCEDURE

1.0 Determination of Cannabinoids in Hemp Using Liquid Chromatography Tandem Mass Spectrometry

2.0 Scope

2.1 This procedure describes the preparation of hemp samples followed by the extraction and quantitation of cannabinoids. Cannabinoid quantitation is accomplished using liquid chromatography tandem mass spectrometry (LC-MS/MS). Cannabinoids that can be analyzed by this procedure include delta-9-tetrahydrocannabinol (Δ^9THC), tetrahydrocannabinolic acid A (THCA), cannabidiol (CBD), and cannabinol (CBN).

3.0 Referenced Documents

3.1 Public Chapter No. 87, Senate Bill No. 357, State of Tennessee, Approved 4 April 2019.

3.2 “Decarboxylation Study of Acidic Cannabinoids: A Novel Approach Using Ultra-High-Performance Supercritical Fluid Chromatography/Photodiode Array-Mass Spectrometry,” Wang, M.; Wang, Y.-H.; Avula, B.; Radwan, M.; Wanas, A.; van Antwerp, J.; Parcher, J.; ElSohly, M.; Khan, I. *Cannabis and Cannabinoid Research*, 2016, 1.1, 262-271.

3.3 “Hemp as an Agricultural Commodity,” Johnson, R., Congressional Research Service, 22 June 2018.

3.4 Safety Data Sheets.

3.5 Technical Services Safety Manual, current revision.

3.6 TecSer 122, *Sample Management*, current revision.

3.7 TecSer 124, *Customer Communications*, current revision.

3.8 “GOOD Test Portions: Guidance On Obtaining Defensible Test Portions,” Laboratory Sampling Working Group, Association of American Feed Control Officials; FASS: Champaign, Illinois, June 2018.

3.9 TecSer 108, *Control Charts, Uncertainty of Measurement*, current revision.

3.10 “The Cannabinoid Content of Legal Cannabis in Washington State Varies Systematically Across Testing Facilities and Popular Consumer Products,” Zoorob, M.; Jikomes, N. *Scientific Reports*, 2018, 8:4519, 1-15.

3.11 "Hemp Sample Preparation," Biochemistry Laboratory SOP No.: PT-LBOP-014, Colorado Department of Agriculture, Sept. 5, 2014.

4.0 Terminology

4.1 Cannabinoid, n – a member of a large family of molecules that act on receptors belonging to the endocannabinoid system

4.1.1 Discussion: Δ^9THC, THCA, CBD, and CBN all belong to this family. Their chemical structures can be found in Attachment 4.

4.2 Delta-9-tetrahydrocannabinol (Δ^9THC), n – the principal psychoactive cannabinoid found within plants of the *Cannabis* genus

4.3 Tetrahydrocannabinolic acid A (THCA), n – a non-psychoactive cannabinoid that undergoes decarboxylation to form Δ^9THC

4.4 Cannabidiol (CBD), n – a non-psychoactive structural isomer of Δ^9THC

4.5 Cannabinol (CBN), n – a non-psychoactive oxidation product of Δ^9THC

4.6 Hemp, n – the plant *Cannabis sativa L.* and any part of the plant

4.6.1 Discussion: State law limits the concentration of certain cannabinoids in hemp on a percent dry weight basis. See Reference 3.1.

4.6.2 Discussion: Weight is a force that is the product of mass and acceleration. Sample weights are determined using scales while sample masses are determined using balances, such as those employed in this procedure. Results determined on a percent dry weight basis are equivalent to those obtained on a percent dry mass basis:

$$\%\ by\ Dry\ Weight = \frac{Weight\ of\ Analyte}{Weight\ of\ Dry\ Sample} * 100\%$$

$$= \frac{(Mass\ of\ Analyte)(Gravitational\ Acceleration)}{(Mass\ of\ Dry\ Sample)(Gravitational\ Acceleration)} * 100\%$$

$$= \frac{Mass\ Analyte}{Mass\ Dry\ Sample} * 100\%$$

$$= \%\ by\ Dry\ Mass$$

4.7 Total THC, n – the weighted sum of Δ^9THC and THCA in a sample

4.7.1 Discussion: The calculation for total THC is described in Section 16.7 and takes into account the mass loss of THCA during decarboxylation.

4.8 Decarboxylation, n – a temperature-dependent decomposition reaction resulting in the loss of a molecule of CO_2

4.8.1 Discussion: For additional information regarding the decarboxylation of THCA to Δ^9THC, see Reference 3.2.

4.9 Comminute, v – to reduce to small particles by crushing, chopping, grinding, etc.

4.10 Test portion, n – the mass of prepared hemp sample subsampled for extraction

4.11 Liquid chromatography tandem mass spectrometry (LC-MS/MS), n – an analytical technique that combines chromatographic separation with mass selection

4.11.1 Discussion: During the liquid chromatography portion of the technique, analytes are separated by retention time according to analyte interactions with stationary and mobile phases. This is coupled to a mass spectrometer with three sequential stages (mass selection of the precursor ion, fragmentation, and mass selection of product ions) prior to detection. The most intense product ion is typically used for quantitation, while a second product ion is used for analyte confirmation. However, if the most intense ion is affected by the presence of an interfering ion, then a second product ion may be used for quantitation.

4.12 Relative standard deviation (RSD), n – the quotient of the standard deviation of an analyte's response over replicate injections (the dividend, σ) and the average analyte response of the same replicate injections (the divisor, μ), mathematically represented as RSD = σ / μ; see Section 12.2

4.12.1 Discussion: RSD provides information on the stability of the instrument, from the injector to the detector for the analyte in question.

4.13 Universal Formula, n – a formula used to determine the concentration of an individual analyte in the original sample

4.13.1 Discussion: This formula is based on the dry sample mass taken for extraction, extraction volume, aliquots diluted or concentrated, injection volumes of standard and sample solutions, instrument response, and standard curve. See Section 16.3.

4.14 Instrument Limit of Quantitation (iLOQ), n – the lowest concentration of an analyte that can be reliably quantitated in a standard injected into the instrument; see Section 16.8

4.15 Instrument Limit of Detection (iLOD), n – the lowest concentration of an analyte that can be injected into the instrument and distinguished from an absence of the same analyte; see Section 16.8

4.16 Method Limit of Quantitation (mLOQ), n – the lowest concentration of an analyte that can be reliably quantitated in an unknown sample; see Section 16.8

4.17 Method Limit of Detection (mLOD), n – the lowest concentration of an analyte that can be distinguished from an absence of the same analyte in an unknown sample; see Section 16.8

4.18 Running standard deviation (RnSD), n – the standard deviation of percent recovery based on the most recent twenty-eight points on an accuracy control chart

4.18.1 Discussion: This provides information on the uncertainty associated with an analytical method across independent analyses. See Section 17.1.

4.19 Units: 1 µg/g = 1 ppm

10,000 ppm = 1%

4.20 Abbreviations:

Table 1. Abbreviations

AcOH	Acetic acid
CAN 1	Cannabinoid Mix 1
CAN 2	Cannabinoid Mix 2
CASRN	Chemical Abstracts Service Registry Number
CBD	Cannabidiol
CBN	Cannabinol
CoA	Certificate of Analysis
HPLC	High-performance liquid chromatography
iLOD	Instrument Limit of Detection
iLOQ	Instrument Limit of Quantitation
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MeOH	Methanol
mLOD	Method Limit of Detection
mLOQ	Method Limit of Quantitation
QC	Quality control
RnSD	Running standard deviation
RSD	Relative standard deviation
SDS	Safety Data Sheet
THCA	Tetrahydrocannabinolic acid A
Δ^9THC	Delta-9-tetrahydrocannabinol

4.21 Acceptable hemp THC level means that when a laboratory tests a sample, it must report the total delta-9 THC content concentration level on a dry weight basis and the measurement of uncertainty. The acceptable level is when the application of the measurement of uncertainty to the reported total delta-9 THC content concentration level on a dry weight basis produces a distribution range that includes 0.3 or less.

5.0 Summary of Method

5.1 Customers request specific cannabinoids for quantitation. Cannabinoids that may be requested include Δ^9THC, THCA, CBD, and CBN. A report of total THC may also be requested which requires both Δ^9THC and THCA to be analyzed.

5.2 Customer request dictates the method of sample preparation. Options that may be requested include using samples as received, oven-drying samples, or air-drying samples. For air-dried samples, a correction factor for moisture is determined which is used to calculate the final results.

5.3 Cannabinoids are extracted from test portions of prepared hemp samples by adding methanol and shaking the samples on a horizontal orbital shaker. Extracts are subsequently centrifuged, and an aliquot of the supernatant is taken for dilution. A portion of this diluted extract is transferred into an autosampler vial for analysis by LC-MS/MS.

5.4 QC samples are analyzed alongside unknown samples to demonstrate statistical control of the procedure.

6.0 Significance and Use

6.1 Hemp is an agricultural commodity that has been historically cultivated for inclusion in a variety of consumer products, including food, nutritional supplements, paper, personal care products, and textiles (see Reference 3.3). In the United States, restrictions on its growth have been recently relaxed, but it is still subject to significant oversight. State law currently limits the amount of Δ^9THC in hemp to no more than 0.3% by dry weight (see Reference 3.1).

6.2 This technical procedure can be used to determine the cannabinoid profile of plant samples. The results obtained using this method are provided to regulatory officials within the Tennessee Department of Agriculture.

7.0 Interferences

7.1 LC-MS/MS is extremely selective, and no interference has been found in this procedure to date. If interference does occur (i.e. same precursor ion, same product ion, and same retention time) quantitation can be accomplished using the second product ion.

8.0 Apparatus

8.1 Refrigerator (1°C to 5°C)

8.2 Freezer (-10°C to -20°C)

8.3 Balance, analytical

8.4 Balance, top-loading

8.5 Positive displacement pipette with tips of various sizes (0.1 mL to 25 mL)

8.4.1 Discussion: All volume-critical pipetting in this procedure is performed using positive displacement pipettes.

8.6 Vortex mixer with adjustable speed setting

8.7 Spatulas

8.8 Centrifuge, refrigerated

8.9 Centrifuge tubes, plastic, 50 mL, can withstand 3500 ×g

8.10 Graduated cylinders, class A (50 mL, 250 mL, 500 mL)

8.11 LC-MS/MS system with gradient pump and autosampler

8.12 HPLC column, C18, 2.1 x 150 mm, 3.5 µm

8.13 Guard column cartridge holder

8.14 Guard column, C18 guard cartridge, 4 x 2.0 mm

8.15 Volumetric flasks, class A, 10 mL

8.16 Autosampler vials, amber, glass, with caps

8.17 Pasteur pipette, disposable, 5¾"

8.18 Horizontal orbital shaker

8.19 Oven

8.20 Aluminum foil

8.21 Desiccator cabinet, amber

8.22 Sieve, No. 8 or smaller mesh screen

8.23 Bottles, amber

8.24 Vials, amber, 12 mL

8.25 Bottle top dispenser, 100 mL

8.26 Bottles, 1 L, for mobile phase

8.27 Discussion: Example vendor information for some apparatus can be found in Attachment 5.

9.0 Reagents and Materials

9.1 Methanol (MeOH), HPLC grade

9.2 Acetic Acid (AcOH), glacial, HPLC grade

9.3 Water, LC-MS grade

9.4 Δ^9THC (CASRN: 1972-08-3) standard, approximately 1000 µg/mL in MeOH or acetonitrile

9.5 THCA (CASRN: 23978-85-0) standard, approximately 1000 µg/mL in MeOH or acetonitrile

9.6 CBD (CASRN: 13956-29-1) standard, approximately 1000 µg/mL in MeOH or acetonitrile

9.7 CBN (CASRN: 521-35-7) standard, approximately 1000 µg/mL in MeOH or acetonitrile

9.8 Dilution Solvent – MeOH:H_2O (1:1, v/v)

9.8.1 Example preparation: Add 250 mL MeOH into a 500 mL graduated cylinder. Dilute to the 500 mL mark with water. Use or discard within six months of preparation.

9.9 HPLC Mobile Phase A – 0.1% Acetic Acid: 10% MeOH: 90% water

9.9.1 Example preparation: Add 50 mL MeOH into a 500 mL graduated cylinder. Pipet 0.5 mL glacial acetic acid into the same graduated cylinder. Dilute to the 500 mL mark with water. Transfer to a 1 L bottle and mix. Use or discard within six months of preparation.

9.10 Oregano, dried

9.11 Discussion: Example vendor information for some reagents and materials can be found in Attachment 5.

10.0 Hazards and Precautions

10.1 Consult Safety Data Sheets (SDSs) (see Reference 3.4) for hazards associated with the use of solvents and other materials.

10.2 Wear protective gloves, safety glasses, lab coat, and any other personal protective equipment specified in the Safety Data Sheets (Reference 3.4) and Technical Services Safety Manual (Reference 3.5).

10.3 The cannabinoid profile of a hemp sample can be altered by exposure to air, heat, and/or light. THCA can decarboxylate to form Δ^9THC, and Δ^9THC can oxidize to form CBN (see Reference 3.2). Ensure that samples are stored in a dark cabinet away from sources of heat and that samples are only oven-dried if specified by customer request.

11.0 Sampling and Sample Preparation

11.1 Sampling and sample receipt relating to the Technical Services Laboratory are described in Reference 3.6.

11.1.1 Samples are collected by regulatory inspectors. These personnel are external to the Technical Services Laboratory.

11.1.2 Samples are inspected by laboratory personnel upon receipt. Any questions regarding the suitability of a sample for analysis are relayed to the Food Residue supervisor, who may in turn contact the customer and the management as needed. Customer communications are documented according to Reference 3.7.

11.1.3 After receipt by the laboratory, hemp samples are to be stored in a dark, secure cabinet or similar holding area away from sources of heat.

11.2 Prior to analyzing a sample, note the method of sample preparation requested by the customer. Any questions that arise are to be relayed to the Food Residue supervisor, who may in turn contact the customer and the management as needed. Customer communications are documented according to Reference 3.7.

11.3 Prepare each sample following the customer request. The customer may request the sample be prepared in accordance with any of the following procedures.

11.3.1 Pre-dried, use samples as received (see Attachment 1)

11.3.2 Oven-dry samples (see Attachment 2)

11.3.3 Air-dry samples (Attachment 3)

11.3.4 Discussion: Customers may request other methods of sample preparation. Document requests following the requirements in Reference 3.7. Failure to adhere to the customer request for sample preparation could significantly impact the analysis in terms of both identity and concentration of cannabinoids found.

12.0 Preparation of Apparatus

12.1 The preparation of the LC-MS/MS system is dependent upon the number and identity of analytes requested for analysis by the customer.

12.1.1 For analyses in which only Δ^9THC is requested, use the instrument parameters found in Attachment 6.

12.1.2 For analyses in which any additional analytes are requested, use the instrument parameters found in Attachment 7.

12.2 Prior to injecting working standards and samples, determine the RSD. This is accomplished by replicate injections of a single solution containing all analytes of interest at a concentration in the midrange of the standard curve. The use of five replicates is acceptable, but additional injections may be incorporated at the analyst's discretion.

13.0 Calibration and Standardization

13.1 A CoA (Certificate of Analysis) for a reference standard is required to be supplied by the vendor before the material is used. Cannabinoid concentrations stated on CoAs are to be employed in the calculation of cannabinoid concentrations in dilutions made from stock reference standards. Store reference standards in accordance with their CoAs. If storage conditions are unspecified on the CoA, store the reference standard in a freezer.

13.2 Cannabinoid intermediate standards are prepared to include the analytes specified by customer request. Additional analytes besides those specified by customer request may be included in an intermediate standard used for an analysis. For example, CBD may be included in an intermediate standard where only Δ^9THC has been requested by the customer.

13.2.1 Cannabinoid Mix 1 (CAN 1): Pipet 0.05 mL of the stock reference cannabinoid standard(s) into a 10 mL volumetric flask as needed to fulfill customer requests. Dilute to volume with MeOH and mix by inversion. Transfer the solution to an amber vial for storage, and clearly label the vial with all pertinent information, including the identity of each cannabinoid included in the mix. Each cannabinoid used is approximately 5 µg/mL in solution.

13.2.2 Cannabinoid Mix 2 (CAN 2): Pipet 1 mL of Cannabinoid Mix 1 (see above) into a 10 mL volumetric flask. Dilute to volume with MeOH and mix by inversion. Transfer the solution to an amber vial for storage, and clearly label the vial with all pertinent information, including the identity of each cannabinoid included in the mix. Each cannabinoid used is approximately 0.5 µg/mL in solution.

13.2.3 Store intermediate standards in a refrigerator or freezer; use or discard intermediate standards within one month of preparation.

13.3 Prepare working standards in autosampler vials using Dilution Solvent (see Section 9.8) and Cannabinoid Mixes 1 and 2.

13.3.1 Pipet 1 mL of Dilution Solvent into each autosampler vial designated to contain a working standard.

13.3.2 Pipet the volume of the appropriate Cannabinoid Mix into each autosampler vial designated to contain a working standard as specified in Table 2.

13.3.3 Cap each working standard and mix by vortexing.

13.3.4 Concentrations listed in Table 2 are approximate. Exact concentrations are to be calculated using the information supplied on the CoA(s) of the stock reference cannabinoids used to prepare CAN 1 and CAN 2.

Table 2. Preparation of working standards

Working Standard	Volume of Intermediate Standard (mL)	Intermediate Standard	Final Volume (mL)	Concentration (µg/mL)
W1	0.025	CAN 2	1.025	0.0122
W2	0.050	CAN 2	1.050	0.0238
W3	0.100	CAN 2	1.100	0.0455
W4	0.175	CAN 2	1.175	0.0745
W5	0.250	CAN 2	1.250	0.1000
W6	0.350	CAN 2	1.350	0.1296
W7	0.035	CAN 1	1.035	0.1691
W8	0.050	CAN 1	1.050	0.2381

14.0 Procedure

14.1 Using an analytical balance, weigh 0.20 ± 0.01 g of prepared hemp sample into a 50 mL centrifuge tube, and record the mass used to 4 decimal places.

14.1.1 Discussion: Hemp is a heterogeneous material. Practice good subsampling techniques to ensure a representative portion is selected for extraction. Additional information regarding good subsampling techniques can be found in Reference 3.8.

14.1.2 Discussion: Moisture determination is necessary for samples that are dried at a low temperature or air-dried at ambient temperature. Follow step 3 of Attachment 3. The mass of the test portion taken for extraction will be corrected for moisture in the final calculations (see Section 16.6.2).

14.2 Using an analytical balance, prepare a method blank by weighing 0.20 ± 0.01 g of oregano into a 50 mL centrifuge tube. Record the actual mass used to 4 decimal places and treat this as an unknown sample.

14.3 For each set of ten unknown samples or fewer, prepare duplicate QC spikes.

14.3.1 Using an analytical balance, weigh 0.20 ± 0.01 g of oregano into two 50 mL centrifuge tubes. Record the actual masses used to 4 decimal places.

14.3.2 Pipet duplicate aliquots of stock reference standards onto the spike samples, using one of the volumes listed in Column 1 of Table 3. These QC samples should be spiked using stock reference standards that represent each analyte requested by the customer. The concentrations listed on the CoAs of the stock reference standards should be used to calculate spiking concentrations.

14.3.3 Vortex the mixture for approximately 30 s and allow the spiking solution(s) to remain in contact with the matrix for 20 to 30 minutes before adding any extraction solvent.

Table 3. Preparation of spike samples

Volume of Each Stock Reference Standard	Cannabinoid Concentration in Stock	Mass of Sample	Concentration of Each Cannabinoid in Spiked

Spiked (mL)	Reference Standard (µg/mL)	Spiked (g)	Sample (%)
0.150	1000	0.2000	0.075
0.200	1000	0.2000	0.100
0.250	1000	0.2000	0.125
0.300	1000	0.2000	0.150

14.4 Dispense 40 mL of MeOH into each of the centrifuge tubes using either a 50 mL graduated cylinder or positive displacement pipette.

14.5 Vortex tubes for approximately 30 seconds, making sure the contents of each tube are well mixed. Shake each tube by hand for approximately 2 minutes.

14.6 Place the unknown sample(s), blank, and spikes on a horizontal orbital shaker. Cover tubes with a towel and shake them for 20 minutes at 500 rpm.

14.7 Centrifuge the tubes at 3500 ×g at 4ºC for 10 minutes.

14.8 A final dilution is made for each unknown sample, blank, and spike. Label a 12 mL amber vial for each dilution, and pipet 10.0 mL of Dilution Solvent into vial. Pipet 50 µL of each supernatant from Section 14.7 into its respective vial. Mix by shaking vials by hand and/or vortexing.

14.9 Using a Pasteur pipette, transfer approximately 1 to 1.5 mL of the final dilution into labelled autosampler vials. Samples are now ready to be analyzed by LC-MS/MS.

15.0 Demonstration of Statistical Control

15.1 For individual analytes such as Δ^9THC, accuracy and precision control charts are constructed as required in TecSer 108 (see Reference 3.9) and are maintained in a shared network folder.

15.2 Total THC is a mathematical combination of Δ^9THC and THCA, so a separate control chart is not constructed for total THC.

16.0 Calculations

16.1 The values used in the calculations presented in this and the following section are purely for example. These values do not necessarily represent anticipated or typical results. Actual values obtained from an analysis may vary.

16.2 Concentration of Individual Analytes in Final Dilution (or Concentration from Curve)

16.2.1 A linear regression curve is generated based on the concentration, *x*, and instrument response, *y*, of the working standards

$$y = mx + b$$

where *m* is the slope and *b* is the y-intercept. The concentration of an analyte in the final dilution can be found by solving for *x* in its respective

regression curve and using the instrument response, y, for that analyte in the final dilution:

$$x = \frac{(y - b)}{m}$$

16.2.2 Example scenario: The instrument response, or peak area, for Δ^9THC in a sample was found to be 7480531. The slope and y-intercept for the regression equation in the analysis were 8.8958×10^7 and 1.207×10^6, respectively. The concentration of Δ^9THC in the final dilution can be found by the following:

$$x = \frac{(7480531 - 1.207 * 10^6)}{\frac{8.8958 * 10^7}{\mu g/mL}} = 0.07052\ \mu g/mL$$

16.3 Concentration of Individual Analytes Found in Spike Samples

16.3.1 The Universal Formula is used to calculate the concentration of an analyte in a sample:

Concentration of an analyte in sample =

$$\frac{Conc\ from\ Curve\ \left(\frac{\mu g}{mL}\right) * Vol\ Standard\ Injected\ (\mu L) * Final\ Vol\ (mL)}{Sample\ Mass\ Extracted\ (g) * Vol\ Sample\ Injected\ (\mu L) * Aliquot\ Fraction}$$

Where,

$$Aliquot\ Fraction = \frac{Aliquot\ Used\ to\ Concentrate\ or\ Dilute}{Total\ Extraction\ Volume}$$

If no aliquot is used, 1/1, i.e. 1, is entered.

16.3.2 Example scenario: 0.1975 g of a spiked sample was extracted with 40.00 mL of MeOH. 50.0 µL of the extract was diluted with 10.00 mL of Dilution Solvent. 15.0 µL of each working standard was injected to prepare the curve, and 15.0 µL of the final dilution of the sample was injected for analysis. The concentration of Δ^9THC from the curve was found to be 0.07052 µg/mL for the spiked sample. The concentration of Δ^9THC in the spike sample is then found using the Universal Formula:

$$\frac{\left(0.07052\ \frac{\mu g}{mL}\right)(15.0\ \mu L)(10.05\ mL)}{(0.1975\ g)(15.0\ \mu L)\left(\frac{0.0500\ mL}{40.00\ mL}\right)}$$

$$= 2871\ ppm * \frac{(1\%)}{(10{,}000\ ppm)}$$

$$= 0.287\%\ \Delta^9 THC$$

16.4 Spiking Level (or Spiking Concentration)

16.4.1 Spiking levels of individual analytes are calculated using the following formula. Exact concentrations listed on CoAs are used.

$$\frac{(Volume\ of\ Reference\ Standard\ Spiked)(Conc\ in\ Reference\ Standard)}{Sample\ Mass}$$

16.4.2 Example scenario: 0.200 mL of Δ^9THC reference standard was spiked onto a 0.1975 g control sample of dried oregano. The CoA for this reference material listed a Δ^9THC concentration of 1003 µg/mL.

$$Spike\ Level = \frac{(0.200\ mL)(1003\ \mu g/mL)}{0.1975\ g}$$

$$Spike\ Level = 1015.7\ ppm * \frac{1\%}{10{,}000\ ppm} = 0.102\%\ \Delta^9 THC$$

16.5 Spike Recovery

16.5.1 Spike recoveries are calculated by subtracting any analyte found in the method blank from the concentration found in the spike sample and comparing that difference to the spike level:

$$\frac{(Concentration\ in\ Spike - Concentration\ in\ Method\ Blank)}{(Spike\ Level)} * 100\%$$

16.5.2 Example scenario: 0.09665% Δ^9THC was found in a spike sample. No Δ^9THC was detected in the method blank. The spike level was 0.102%. The spike recovery is calculated to be

$$\frac{(0.09665\% - 0\%)}{(0.102\%)} * 100\% = 94.8\%$$

16.6 Concentration of Individual Analytes in Unknown Samples

16.6.1 Pre-dried and oven-dried samples

16.6.1.1 Individual cannabinoid concentrations can be calculated by following the calculations under Sections 16.2 and 16.3.

16.6.2 Air-dried samples

16.6.2.1 Individual cannabinoid concentrations can be calculated by following the calculations under Section 16.2 and 16.3; however, an additional correction factor is required to compensate for the moisture content of the test portion:

$$Sample\ Mass\ Extracted = Test\ Portion\ Mass * (1 - Moisture\ Fraction)$$

Where the test portion mass comes from Section 14.1 and the moisture fraction is determined using the data obtained in step 3 of Attachment 3:

$$Moisture\ Fraction = \frac{(Initial\ Sample\ Mass - Final\ Sample\ Mass)}{Initial\ Sample\ Mass}$$

16.6.2.2 Example scenario:

A sample received by the lab was designated for air-drying. After the sample was deemed dry enough to be worked through a sieve, the sample was comminuted. A 0.2041 g test portion of the comminuted sample was taken for extraction. This was extracted with 40.0 mL MeOH, and 0.0500 mL of the extract was diluted with 10.00 mL Dilution Solvent to prepare the final dilution for injection.

The moisture fraction of the test portion was determined using a separate portion of the comminuted sample. The mass of an aluminum pan was recorded as 1.0015 g. Comminuted sample was added to this pan until their combined mass was 2.0035 g. After heating until a consistent dry mass was achieved, the final combined mass of the pan and sample was recorded as 1.8921 g.

15.0 µL injections were used for sample injections as well as for standards, and the concentration of Δ^9THC from the curve was found to be 0.05739 µg/mL.

Calculation of the Moisture Fraction for this sample:

$$\frac{(2.0035\ g - 1.0015g) - (1.8921g - 1.0015g)}{(2.0035g - 1.0015g)} = 0.1112$$

Calculation of Δ^9THC in this sample:

$$\frac{\left(0.05739\ \frac{\mu g}{mL}\right)(15.0\ \mu L)(10.05\ mL)}{[(0.2041g)(1 - 0.1112)](15.0\ \mu L)\left(\frac{0.0500\ mL}{40.0\ mL}\right)}$$

$$= 2544\ ppm * \frac{(1\%)}{(10{,}000\ ppm)} = 0.254\%\ \Delta^9 THC$$

16.7 Concentration of Total THC in Samples

16.7.1 Total THC in a sample is calculated for all sample types (pre-dried, oven-dried, and air-dried) using the following equation (see Reference 3.10):

$$Conc\ Total\ THC = (Conc\ THCA * 0.877) + Conc\ \Delta^9 THC$$

This equation is derived from the molecular weights of Δ^9THC and THCA to compensate for the mass lost during the decarboxylation of THCA to Δ^9THC:

$$\frac{MW\ \text{of}\ \Delta^9 THC}{MW\ of\ THCA} = \frac{314.5}{358.5} = 0.877$$

16.7.2 Example scenario: An unknown sample was found to be 0.167% Δ^9THC and 0.138% THCA. The total THC content is found to be:

$$(0.138\% * 0.877) + 0.167\% = \ 0.288\%\ Total\ THC$$

16.8 Limits of Quantitation and Detection

16.8.1 Individual Analytes

16.8.1.1 The iLOQ for an analyte is the lowest concentration of that analyte used to prepare the standard/calibration curve, and the iLOD of an analyte is a fraction of the iLOQ, determined at the analyst's discretion.

The mLOQ and mLOD for individual analytes are found by applying the Universal Formula to the iLOQ and iLOD, respectively, in place of a concentration from the curve.

16.8.1.2 Example scenario: Approximately 0.20 g test portions were taken for extraction with 40.0 mL of MeOH, and final dilutions were prepared for each sample by diluting 50 µL of extract with 10.00 mL of Dilution Solvent. 15.0 µL injections were specified for samples as well as working standards, and the lowest concentration working standard used to prepare the Δ^9THC calibration curve was 0.0122 µg/mL Δ^9THC. The analyst determined that a concentration of approximately half the lowest standard could still be differentiated from the background noise for Δ^9THC.

iLOQ

$$Lowest\ Concentration\ on\ the\ Curve = 0.0122 \frac{\mu g}{mL} \Delta^9 THC$$

iLOD

$$iLOQ * 0.50 = \ 0.0122 \frac{\mu g}{mL} * 0.50 = 0.0061 \frac{\mu g}{mL} \Delta^9 THC$$

mLOQ

$$\frac{\left(0.0122 \frac{\mu g}{mL}\right)(15.0\ \mu L)(10.05\ mL)}{(0.20\ g)(15.0\ \mu L)\left(\frac{0.050\ mL}{40.0\ mL}\right)}$$

$$= 490\ ppm * \frac{(1\%)}{(10{,}000\ ppm)} = 0.049\%\ \Delta^9 THC$$

mLOD

$$\frac{\left(0.0061\ \frac{\mu g}{mL}\right)(15.0\ \mu L)(10.05\ mL)}{(0.20\ g)(15.0\ \mu L)\left(\frac{0.050\ mL}{40.0\ mL}\right)}$$

$$= 245\ ppm * \frac{(1\%)}{(10{,}000\ ppm)} = 0.025\%\ \Delta^9 THC$$

16.8.2 Total THC

16.8.2.1 As total THC is the mathematical combination of two individual analytes, no iLOQ or iLOD can be determined. Thus, no mLOQ or mLOD can be reported for total THC.

17.0 Measurement of Uncertainty is reported with the test results to determine the acceptable hemp THC level by using the following appropriate methods and procedures.

17.1 Method Uncertainty

17.1.1 Individual Analytes

17.1.1.1 The method uncertainty for an individual cannabinoid is defined by ± 2 RnSD, where RnSD is calculated using the most recent twenty eight points on the cannabinoid's respective accuracy control chart(s) (see Section 15.1).

17.1.2 Total THC

17.1.2.1 The method uncertainty for total THC is calculated by first propagating the uncertainties of Δ^9THC and THCA, defined by their RnSDs, σ, through their linear combination:

$$\sigma_{Total\ THC} = \sqrt{(0.877 * \sigma_{THCA})^2 + (\sigma_{\Delta^9 THC})^2}$$

17.1.2.2 The method uncertainty for total THC is defined by ± 2 $\sigma_{\text{Total THC}}$.

17.1.2.3 Example scenario: A report of total THC was requested for a set of samples. The RnSDs for Δ^9THC and THCA for this analysis were 5.4% and 6.7%, respectively. The method uncertainty for total THC would be:

$$= 2 * \sqrt{(0.877 * \sigma_{THCA})^2 + (\sigma_{\Delta^9 THC})^2}$$

$$= 2 * \sqrt{(0.877 * 6.7\%)^2 + (5.4\%)^2}$$

$$= 16\%$$

17.2 Result Uncertainty

17.2.1 The uncertainty of any given result is the method uncertainty of that result multiplied by the result itself. This applies to individual analytes as well as total THC.

17.2.2 Example scenario: A result of 0.284% Δ^9THC was found. The running standard deviation for Δ^9THC for this analysis was 5.4%, or 0.054. The uncertainty of the result would be:

$$= (Method\ Uncertainty)(Result)$$

$$= (2 * RnSD)(Result)$$

$$= (2 * 0.054)(0.284\%\ \Delta^9 THC)$$

$$= 0.031\%\ \Delta^9 THC$$

17.2.3 Example scenario: A result of 0.256% total THC was found. The running standard deviations for Δ^9THC and THCA for this analysis were 5.4% and 6.7%, respectively. The uncertainty of the result would be:

$$= (Method\ Uncertainty)(Result)$$

$$= (2 * \sigma_{Total\ THC})(Result)$$

$$= \left(2 * \sqrt{(0.877 * \sigma_{THCA})^2 + (\sigma_{\Delta^9 THC})^2}\right)(Result)$$

$$= \left(2 * \sqrt{(0.877 * 0.067)^2 + (0.054)^2}\right)(0.256\%\ Total\ THC)$$

$$= (0.16)(0.256\%\ Total\ THC)$$

$$= 0.041\% \, Total \, THC$$

Preparation of Pre-Dried Samples

When a customer specifies that a sample submitted to the lab is dry and is to be used as received, the sample preparation procedure in this attachment is followed.

1 Drying

1.1 If the sample has been specified as dry by the customer, no additional drying is needed, and no moisture determination is necessary.

2 Sample Comminution

2.1 Place a sieve atop a fresh piece of aluminum foil.

2.2 Add a portion of the hemp sample into the sieve, removing stems and seeds as able. Mostly leaf and flower material should remain.

2.3 Work the sample through the sieve.

2.4 Continue adding portions of the dried sample until the entire sample has been worked through the sieve or sorted out as extraneous material, e.g. stems and seeds.

2.5 Fold extraneous material inside of the aluminum foil pan, and store it with the evidence bag in a secure cabinet.

2.6 Place the sieve aside, and continue to work the sieved sample by hand until it achieves a relatively uniform, fine consistency.

2.7 Carefully transfer the comminuted sample to an appropriately labelled amber jar, and store the amber jar in a dark, secure cabinet away from sources of heat until ready to proceed with analysis.

Sample Preparation by Oven-Drying

When the customer requests that a sample be prepared by oven-drying at high temperature, the sample preparation procedure in this attachment is followed. This is adapted from Reference 3.11.

1 Sample Drying

1.1 Fold the edges of a sheet of aluminum foil to form a pan.

1.2 Determine the mass of the aluminum pan using a top-loading balance, and record the mass in the analyst's notebook.

1.3 Spread the entire sample onto the pan.

1.3.1 Discussion: If the sample cannot be spread onto a single pan, the sample may be split into two or more pans. The mass of each pan should be recorded prior to the addition of sample.

1.4 Determine the combined mass of the sample and aluminum pan using a top-loading balance, and record the mass in the analyst's notebook.

1.5 Load the sample and pan into an oven set to 90 ± 5°C, and allow the sample to heat for two hours.

1.6 Remove the sample and pan from the oven, and allow it to cool for at least 30 minutes in an amber desiccator cabinet.

1.7 Determine the combined mass of the sample and pan using a top-loading balance, and record the mass in the analyst's notebook.

1.8 Return the sample and pan to the oven for an additional 15 minutes of heating at 90 ± 5°C.

1.9 Remove the sample and pan from the oven, and allow it to cool for at least 30 minutes in an amber desiccator cabinet.

1.10 Determine the combined mass of the sample and pan using a top-loading balance, and record the mass in the analyst's notebook.

1.11 If the sample mass changes by less than 1% between steps 1.7 and 1.10, the drying is complete. If the change is greater than or equal to 1%, repeat step 1.8 onwards.

2 Sample Comminution

2.1 Once the sample is deemed dry, place a sieve atop a fresh piece of aluminum foil.

2.2 Add a portion of the hemp sample into the sieve, removing stems and seeds as able. Mostly leaf and flower material should remain.

2.3 Work the sample through the sieve.

2.4 Continue adding portions of the dried sample until the entire sample has been worked through the sieve or sorted out as extraneous material, e.g. stems and seeds.

2.5 Fold extraneous material inside of the aluminum foil pan, and store it with the evidence bag in a secure cabinet.

2.6 Place the sieve aside, and continue to work the sieved sample by hand until it achieves a relatively uniform, fine consistency.

2.7 Carefully transfer the comminuted sample to an appropriately labelled amber jar, and store the amber jar in a dark, secure cabinet away from sources of heat until ready to proceed with analysis.

Sample Preparation by Air-Drying

When the customer requests that a sample be prepared by air-drying, the sample preparation procedure in this attachment is followed.

1 Sample Drying

1.1 Samples received by laboratory personnel may vary greatly in moisture content depending upon the sampling conditions. The intention of the drying procedure in this attachment is not to remove all moisture from a sample prior to comminution, but to dry the sample sufficiently such that comminution is performed more easily.

1.2 When a sample is received from a customer, it is stored in a dark cabinet away from sources of heat. Samples are to be inspected for comminution suitability based on the analyst's discretion.

1.2.1 If a sample bag is non-transparent, the sound of dry vegetation cracking when the bag is compressed indicates suitability for comminution. This indirect observation allows tamper-evident seals to remain intact until immediately prior to comminution.

1.2.2 If a sample requires additional drying, it may be stored in dark conditions in a secure area away from sources of heat until deemed sufficiently dry.

2 Sample Comminution

2.1 Once the sample is deemed sufficiently dry, place a sieve atop a fresh piece of aluminum foil.

2.2 Add a portion of the hemp sample into the sieve, removing stems and seeds as able. Mostly leaf and flower material should remain.

2.3 Work the sample through the sieve.

2.4 Continue adding portions of the dried sample until the entire sample has been worked through the sieve or sorted out as extraneous material, e.g. stems and seeds.

2.5 Fold extraneous material inside of the aluminum foil pan, and store it with the evidence bag in a secure cabinet.

2.6 Place the sieve aside, and continue to work the sieved sample by hand until it achieves a relatively uniform, fine consistency.

2.7 Carefully transfer the comminuted sample to an appropriately labelled amber jar, and store the amber jar in a dark, secure cabinet away from sources of heat until ready to proceed with analysis.

3 Moisture Determination

3.1 When a test portion is weighed out for extraction in Section 14.1, weigh out a second test portion to determine the moisture content of the sample at the time of subsampling:

3.1.1 Weigh a labelled aluminum pan on an analytical balance and record the mass to four decimal places in the analyst's notebook.

3.1.2 Add approximately 1 g of the comminuted sample to the aluminum pan using good subsampling techniques.

3.1.2.1 Discussion: Hemp is a heterogeneous material. Practice good subsampling techniques to ensure a representative portion is selected for analysis. Additional information regarding good subsampling techniques can be found in Reference 3.8.

3.1.3 Reweigh the sample and pan on an analytical balance, and record their combined mass to four decimal places in the analyst's notebook.

3.2 Load the sample and pan into an oven at 105 ± 5°C, and allow them to heat overnight.

3.3 Transfer the sample and pan into an amber desiccator cabinet, and allow them to cool for 30 minutes.

3.4 Reweigh the sample and pan on an analytical balance, and record their combined mass to four decimal places in the analyst's notebook.

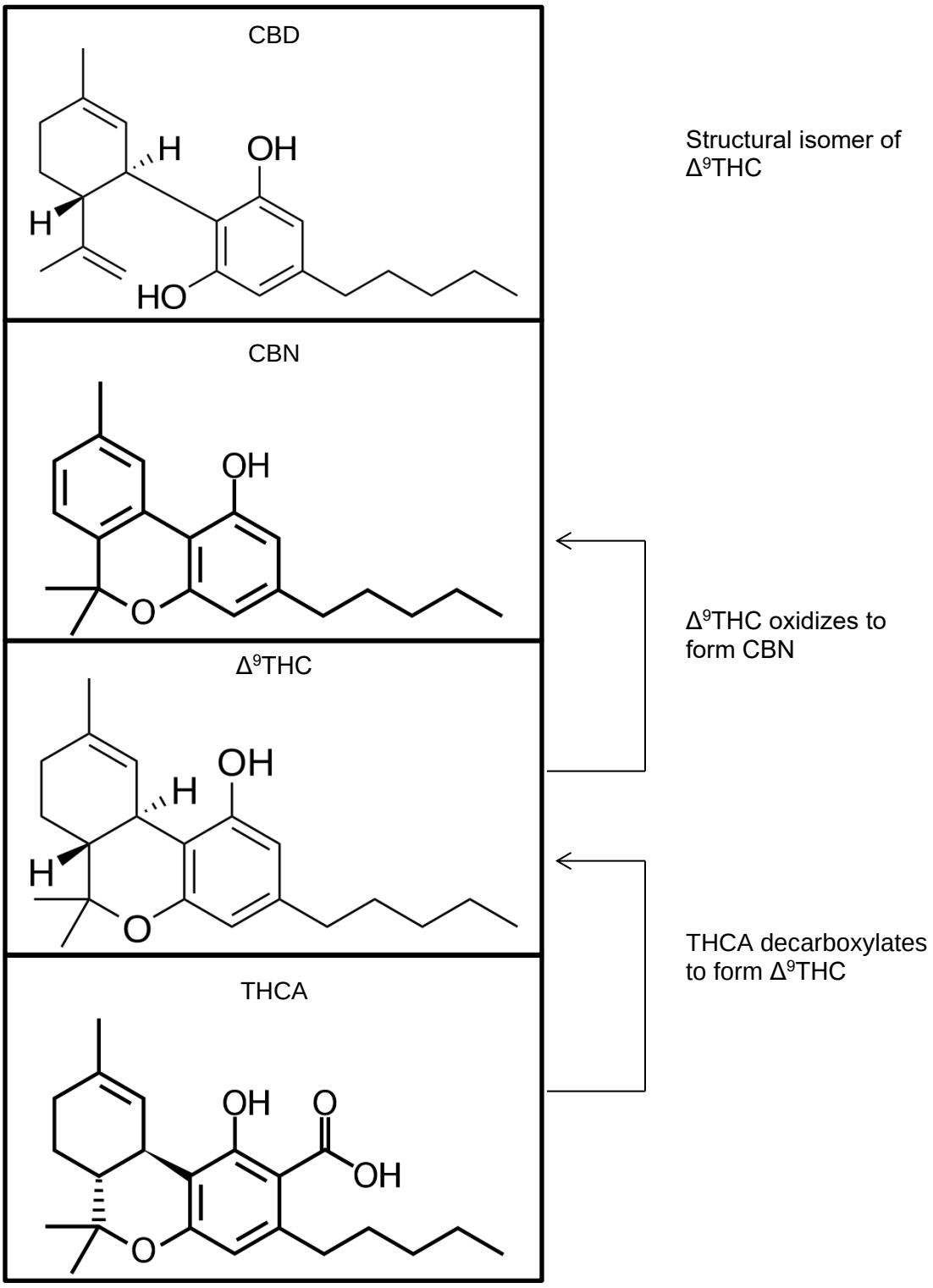
3.5 Return the sample and pan to the oven at 105 ± 5°C for an additional hour of heating.

3.6 Transfer the sample and pan to an amber desiccator cabinet, and allow them to cool for 30 minutes.

3.7 Reweigh the sample and pan on an analytical balance, and record the mass to four decimal places.

3.8 If the sample mass changes by less than 1% between steps 3.4 and 3.7, the drying for moisture determination is complete. If the change is greater than or equal to 1%, repeat step 3.5 onwards.

Structures of Cannabinoids Covered by This Procedure



Structural isomer of Δ^9THC

Δ^9THC oxidizes to form CBN

THCA decarboxylates to form Δ^9THC

Example Vendor Information

The information provided in the following table serves only as an example of vendor information that can be used for purchasing some of the apparatus, materials, and reagents mentioned in the main body of the document. Equivalent products may be substituted for any or all of the items listed.

Table X5.1: Example vendor information

Positive displacement pipettes	Eppendorf, ER-E3
Centrifuge, refrigerated	Thermo, Sorvall Legend RT+
Centrifuge tubes, 50 mL	VWR, Cat# 21008-240
LC-MS/MS system with gradient pump and autosampler	Dionex UltiMate 3000 with Thermo TSQ Quantum MAX
HPLC column, C18, 2.1x150 mm, 3.5 µm	Fisher, Cat# 50-926-251
Guard column cartridge holder	Phenomenex, Cat# KJ0-4282
Guard column, C18 guard cartridge, 4 x 2.0 mm	Phenomenex, Cat# AJ0-4286
Autosampler vials	Fisher, Cat # 03-377-10D
Pasteur pipette, disposable, 5¾"	Fisher, Cat# 13-678-20A
Methanol, HPLC grade	Fisher, Cat# A454-4
Acetic acid, glacial, HPLC grade	Fisher, Cat# A35-500
Water, LC-MS grade	Fisher, Cat# W6-4
Δ^9THC standard	Fisher, Cat# 06-722-453
THCA standard	Fisher, Cat# NC1056549
CBD standard	Fisher, Cat# NC0758686
CBN standard	Fisher, Cat# NC1583154

Instrument Conditions – Only Δ^9THC Requested

The instrument conditions in this attachment are suggested parameters for use in combination with a Dionex 3000 UltiMate and TSQ Quantum Access MAX LC-MS/MS system. Parameters may need to be adjusted for use with a different LC-MS/MS system or over time.

HPLC Conditions

Column: Agilent Zorbax SB C18, 2.1 x 150 mm, 3.5 µm, or equivalent
Temperature: 30°C
Solvent A: 0.1% AcOH, 10% MeOH, 90% Water
Solvent C: MeOH
Injection Volume: 15 µL
Flow Rate: 0.25 mL/min

Table X6.1: HPLC gradient for Δ^9THC only

Time (minutes)	% C
0	50
1	50
8	90
18	90
18.5	50
22.5	50

Retention time of Δ^9THC ~14.0 minutes

MS/MS Conditions

Table X6.2: MS/MS conditions for Δ^9THC only

	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy	Scan Time (s)	Start Time (minutes)	End Time (minutes)	Tube Lens	Polarity
Δ^9THC	315.355	193.12	22	0.04	12	16	108	+
Δ^9THC (Conf)	315.35	123.07	32	0.05	12	16	108	+

Instrument Conditions – Additional Analytes Requested beyond Δ^9THC

The instrument conditions in this attachment are suggested parameters for use in combination with a Dionex 3000 UltiMate and TSQ Quantum Access MAX LC-MS/MS system. Parameters may need to be adjusted for use with a different LC-MS/MS system or over time.

HPLC Conditions

Column:	Agilent Zorbax SB C18, 2.1 x 150 mm, 3.5 µm, or equivalent
Temperature:	30°C
Solvent A:	0.1% AcOH, 10% MeOH, 90% Water
Solvent C:	MeOH
Injection Volume:	15 µL
Flow Rate:	0.25 mL/min

Table X7.1: HPLC gradient for 4 cannabinoids

Time (minutes)	% C
0	50
1	50
8	90
18	90
18.5	50
22.5	50

Table X7.2: Approximate retention times

Cannabinoid	Approximate Retention Time (minutes)
CBD	12.2
CBN	13.5
Δ^9THC	14.0
THCA	17.0

MS/MS Conditions

Table X7.3: MS/MS conditions for 4 cannabinoids

	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy	Scan Time (s)	Start Time (minutes)	End Time (minutes)	Tube Lens	Polarity
CBD	315.354	193.11	21	0.05	11.45	13.95	94	+
CBD (Conf)	315.353	123.04	31	0.05	11.45	13.95	94	+
CBN	311.323	223.11	20	0.05	12	16	96	+
CBN (Conf)	311.322	293.24	17	0.05	12	16	96	+
Δ^9THC	315.355	193.12	22	0.04	13.55	16.05	94	+
Δ^9THC (Conf)	315.356	123.07	32	0.04	13.55	16.05	94	+
THCA	357.351	313.38	27	0.04	16.5	20.5	98	-
THCA (Conf)	357.350	245.28	36	0.04	16.5	20.5	98	-

ATTACHMENT D

Performance Based Sampling

Background

From 2015 through 2020 the department tested 1600 samples. There were 10 violations during this period. That is a violation rate of .6 percent. (See Table 1.) There are many growers that are not likely to have a violation. Over this period no grain, greens, microgreens, transplants, or research growers had a violation. There are many growers who have proven a track record of no violations as well.

Goal

While focusing on results, flexibility, and efficiency, the department will use the USDA performance-based sampling approach where the method of sampling ensures a confidence level of 95 percent that no more than one percent of the plants in each lot would exceed the acceptable hemp THC level and ensure that a representative sample is collected that is a homogeneous composition of the lot.

Definitions

When used in this plan, unless the context requires otherwise:

- Research institution means an accredited institution of higher learning, a research facility that conducts scientific research on hemp, or any licensed person growing hemp for research purposes, and none of the hemp is intended for commerce.

- Microgreens and greens mean immature hemp seedlings for human consumption that are cut-off above the soil or substrate line and harvested prior to flowering and not more than 14 days after germination. Hemp microgreens are typically between two (2) and (3) inches in height, but not taller than five (5) inches. Greens are no more than ten (10) inches tall and not flowering.

- Transplants mean nonflowering hemp seedlings, rooted cuttings, immature plants produced from tissue culture, or other means of reproduction, which are not harvested but transplanted into a large container or field to mature for harvest. The movement of transplants from their original location to the crop production location is not considered a harvest.

- Fiber, grain, and seed hemp mean hemp grown for the sole purpose of being harvested for fiber, seed, or grain.

- Legacy growers mean producers who have a proven record with TDA that have grown hemp for at least 3 consecutive years, been sampled in each of those years, and those tests were below the acceptable limit.

- Hemp not intended for commerce means hemp that is not sold or transferred to another entity after harvest.

Program

(1) The performance-based sampling methods meet the following criteria as described in (a)(2)(iii)(A) and (B) of 7 CFR §990.3:

a. The performance-based sampling method is included in the State's hemp plan and will be reviewed and approved by USDA.

b. The performance-based method will ensure, at a confidence level of 95 percent and that the cannabis plants tested will not test above the acceptable hemp THC level.

(2) Performance-based sampling does not prevent the department from conducting random records inspections or sampling and testing of any hemp crops from a licensee. The department reserves the right to conduct a records inspection, sample, and test any hemp lot at any time to ensure compliance with the acceptable hemp THC level.

(3) Performance based sampling will include different sampling frequencies and requirements for the following categories of hemp producers:

(a) Hemp Not Intended for Commerce and Hemp Grown for Research.

1. To qualify for alternative sampling methods, producers must provide an application for a research license under Rule 0080-06-28-.03(10).

2. Hemp grown for research shall not enter commerce.

3. Universities and research institutions may self-report results of sampling and testing under the following conditions:

(i) Results regarding research are shared with the public or published on the research institution website.

(ii) The research producer provides the scope and standard operating procedures forproduction of hemp.

(iii) The research producer provides a disposal plan for all hemp produced includingphotographic evidence for verification.

(iv) The research producer allows the department to inspect or audit the above documentation and testing resultson an annual basis.

(v) The research producer reports to FSA as required in the guidelines for reporting license numbers and acreage. The acreage report does not have to be broken down by lots. If the field was planted over several days, the report will include only the average day of planting.

(vi) The faculty member in charge of the research has submitted a criminal background check in the application process.

4. Any non-compliant lots of hemp produced by a university research institution (or research institution)shall be disposed of and reported to the department.

(b) Fiber, Grain, and Seed Hemp and Hemp Grown by Legacy Growers.

1. If producers are harvesting for grain or fiber, there is less need to sample because these strains of cannabis plants are generally below 0.3% THC. For this reason, 50% of these producers will be sampled every year. Producers growing varieties that test above the allowable total THC concentration will be tested every growth cycle will, be required to have subsequent lots of that variety tested every growth cycle, and will be eligible for testing every other year after receiving a test result within the allowable total THC concentration for that variety.

2. If a producer is sampled and the lot fails the pre-harvest test, the producer will have the option to remediate the lot or destroy it.

3. A hemp producer who has met all five of the following compliance history requirements may not be subject to testing in the current year:

   (i) produced hemp for a period of three (3) consecutive years.

   (ii) has had their hemp tested by the department or an authorized testing agent each of those years.

   (iii) all results in each of the previous three (3) years were below the acceptable hemp THC level.

   (iv) are growing the same varieties or cultivars as in the previous three (3) years. (See Table 2.) and

   (v) are growing varieties or cultivars that have not been found in violation. (See Table 3.)

(c) Microgreens, Greens, Transplants, and hemp transplants

1. If hemp producers harvest microgreens, the department shall conduct random testing of these plants to include 25% of all lots produced. Due to extremely low levels of cannabinoids in the very immature plants, the sampling and testing of every harvest of every lot is impractical and unnecessary.

2. The producer shall ensure that the seeds used to produce microgreens are from cannabis varieties that meet the definition of hemp. Hemp microgreen operations shall be subject to random inspections and sampling.

3. Since the definition of hemp greens involves the leaves of very immature plants (less than 10 inches tall) and not flowering, it would be unnecessary to sample every lot of that hemp.

4. The producer shall ensure that the seeds used to produce hemp greens are from cannabis varieties that meet the definition of hemp. Lots harvested for hemp greens shall be subject to random inspections and sampling.

5. The transfer of hemp transplants to the location at which these plants will grow to maturity and from which these plants will be harvested shall not be considered a harvest. Hemp transplants will not be subject to sampling before the plants are transferred to the location at which these plants will grow to maturity and from which these plants will be harvested. However,

the mature crop produced from hemp transplants is subject to sampling and testing.

Table 1.

Year	non-compliant	Samples Taken
2015	0	56
2016	1	39
2017	1	38
2018	2	317
2019	1	950
2020	5	207

Table 2.
Varieties Tested for 3 Consecutive Years

2018 - 2020 Hemp Sample Information							
Sample Name	**Count**	**Lowest Result**	**Highest Result**	**Average Result**	**2018**	**2019**	**2020**
Boax	34	0.039	0.514	0.133	2	26	6
Bubble Gum	3	0.073	0.113	0.093	1	1	1
Cherry	22	0.041	0.186	0.098	3	15	4
Cherry Wine	57	0.057	0.289	0.095	3	38	16
Frosted Lime	24	0.058	0.377	0.147	6	16	2
Siskyou Gold	7	0.058	0.27	0.13	2	4	1
Stout	15	0.039	0.141	0.082	6	7	2
Sweetened	132	0.064	0.443	0.144	26	96	10
Trump 1	11	0.047	0.186	0.092	7	2	2
Young Sim 10	14	0.065	0.258	0.14	1	11	2

The highlighted varieties will require annual testing.

Table 3.

The highlighted varieties will require annual testing.

2018 - 2020 Hemp Sample Information

Sample Name	Count	Lowest Result	Highest Result	Average Result	2018	2019	2020
00CHERRY	1	0.109	0.109	0.109	0	1	0
2Sweetened	1	0.073	0.073	0.073	1	0	0
Abacus	1	0.186	0.186	0.186	0	1	0
Abacus 2.0	1	0.256	0.256	0.256	0	0	1
Abacus Early Bird 2.0	1	0.119	0.119	0.119	0	0	1
AC/DC 1	1	0.114	0.114	0.114	1	0	0
AC/DC x Cherry Wine	1	0.076	0.076	0.076	0	1	0
ACDC	7	0	0	0	0	7	0
Auto Pilot 1	1	0.083	0.083	0.083	0	0	1
AUTOPILOT	2	0.067	0.067	0.067	0	1	1
BC BUD CBD	1	0.058	0.058	0.058	0	0	1
Berry Blossom	5	0.067	0.173	0.129	0	3	2
Big Orange	2	0.058	0.092	0.075	0	0	2
Black Jack	1	0	0	0	0	0	1
Blue Genius	1	0.082	0.082	0.082	0	0	1
Boax	34	0.039	0.514	0.133	2	26	6
BORDEAUX	1	0	0	0	0	1	0
BOXWINE	2	0.136	0.136	0.136	0	2	0
Boxwine S1	1	0.116	0.116	0.116	0	0	1
Bubba Kush	2	0.245	0.245	0.245	0	0	2
Bubble Gum	3	0.073	0.113	0.093	1	1	1
C4	1	0.082	0.082	0.082	0	1	0
C5	1	0.079	0.079	0.079	0	0	1
Cannaboost	15	0.044	0.271	0.105	12	3	0
Carmagnola	3	0.117	0.335	0.231	3	0	0
Carmagnola selezionata	2	0.077	0.17	0.123	2	0	0
Carolina	11	0.057	0.257	0.157	0	10	1

Carolina & Siskiyou Late	1	0	0	0	0	1	0
Carolina Dream	1	0.046	0.046	0.046	0	0	1
CAROLINAPEACH	1	0	0	0	0	1	0
Cats Meow	1	0	0	0	0	1	0
CBD Gold	1	0	0	0	0	0	1
CBD Haze	1	0	0	0	0	1	0
CBDawg	9	0.091	0.306	0.169	6	3	0
CBG	5	0.067	0.096	0.076	0	1	4
CBG 1	1	0.056	0.056	0.056	0	0	1
CBG Gold	1	0.038	0.038	0.038	0	0	1
CBG Mountain Blizzard	1	0.095	0.095	0.095	0	0	1
CBG White	4	0.081	0.108	0.096	0	0	4
CHAR	1	0	0	0	0	1	0
Chardonnay	8	0.079	0.13	0.105	0	6	2
Cherry	22	0.041	0.186	0.098	3	15	4
Cherry Ascenine	1	0.17	0.17	0.17	0	0	1
Cherry Blossom	2	0.064	0.389	0.226	0	0	2
Cherry Blossom 50	1	0.327	0.327	0.327	0	0	1
Cherry Cherry	4	0.283	0.283	0.283	1	3	0
Cherry Citrus	3	0	0	0	0	3	0
Cherry Crisp	1	0	0	0	0	1	0
Cherry Frost	1	0.058	0.058	0.058	0	1	0
Cherry Mom	1	0.126	0.126	0.126	1	0	0
CHERRY TANG	9	0.053	0.492	0.202	1	8	0
Cherry Trump	1	0	0	0	0	0	1
Cherry Wine	57	0.057	0.289	0.095	3	38	16
Cherry Wine II	1	0	0	0	0	1	0
Cherry X Otto II "Sweet"	1	0.111	0.111	0.111	1	0	0
CHERRY5	1	0	0	0	0	1	0
Cherry-Boax	1	0	0	0	0	1	0
Chery Tang	1	0.142	0.142	0.142	1	0	0
Cinco	2	0.127	0.228	0.177	0	0	2
Cisco Gold	2	0	0	0	0	2	0
CISKYOUGOLD	1	0	0	0	0	1	0
Citral	1	0.163	0.163	0.163	0	0	1
Citrus Fire	3	0.066	0.316	0.165	2	0	1
CLUM	1	0	0	0	0	1	0
COBBLER	2	0.079	0.079	0.079	0	1	0
Cold War	1	0.063	0.063	0.063	0	0	1
Composite	2	0.063	0.063	0.063	0	2	0

Composite Sample	4	0.056	0.069	0.062	0	4	0
Delta Wine	3	0.056	0.151	0.103	0	3	0
Double Bubble	4	0.049	0.247	0.116	0	0	4
DRHORN	1	0.304	0.304	0.304	0	1	0
Early Guel	1	0.274	0.274	0.274	0	1	0
Electra	1	0	0	0	0	1	0
Elleta Compana	1	0.039	0.039	0.039	1	0	0
EMAMALIE	1	0	0	0	0	1	0
Endurance	3	0.035	0.107	0.071	2	1	0
Enlightened OG	1	0.063	0.063	0.063	0	0	1
FALL BLOOM	1	0.139	0.139	0.139	0	1	0
Fedora17	1	0.117	0.117	0.117	1	0	0
FIbranova	4	0.051	0.109	0.08	4	0	0
FLORENCE	1	0	0	0	0	1	0
Franklin	42	0.031	0.218	0.105	12	30	0
Frosted Cherry	1	0.075	0.075	0.075	0	1	0
Frosted Lime	24	0.058	0.377	0.147	6	16	2
Futura75	2	0.052	0.078	0.065	2	0	0
Golden kush	2	0.105	0.134	0.119	0	0	2
Goliath	5	0.184	0.184	0.184	0	0	5
Goliath Peach	1	0.154	0.154	0.154	0	0	1
HASH	1	0.091	0.091	0.091	0	1	0
Hawaiian Haze	17	0.07	0.095	0.084	0	17	0
Hemp Hulk	1	0.132	0.132	0.132	0	0	1
HK12	3	0.082	0.424	0.231	3	0	0
Hot Blonde	1	0.145	0.145	0.145	0	0	1
HURRICANE	1	0	0	0	0	1	0
JUPITER	1	0	0	0	0	1	0
Keatts	1	0.083	0.083	0.083	0	1	0
KETNERKUSH	1	0	0	0	0	1	0
Ketner's Kush, CBG	1	0.107	0.107	0.107	0	0	1
KLR1	3	0.058	0.076	0.067	0	2	1
KLR2	1	0	0	0	0	1	0
La Cream	1	0	0	0	0	0	1
Large Marge	1	0.239	0.239	0.239	0	0	1
Late Sue	5	0.064	0.095	0.079	2	3	0
LATIMER	2	0	0	0	0	2	0
Lifter	14	0.055	0.564	0.252	0	12	2
Lindorea	1	0	0	0	0	0	1
Little Giant	1	0.06	0.06	0.06	0	0	1
MAMMOTH	3	0	0	0	0	3	0
Marian Berry	1	0	0	0	0	1	0

Marionberry	2	0.093	0.093	0.093	0	2	0
Merlot	1	0.256	0.256	0.256	0	0	1
Midwest	8	0.056	0.245	3	0	7	1
Mixed Varieties	239	0.053	0.337	0.102	3	222	14
MOJICA	1	0	0	0	0	1	0
Mountain Gold	2	0.142	0.142	0.142	0	0	2
Mountain Mango	9	0.068	0.18	0.108	0	5	4
Naked Lady-Alpine	1	0	0	0	0	0	1
New West Genetic	1	0	0	0	0	0	1
NS-39	1	0	0	0	0	1	0
Orchard Tang	2	0.054	0.054	0.054	0	1	1
ORIGINAL CHERRY	1	0.068	0.068	0.068	0	1	0
Otter	3	0.099	0.863	0.481	2		
Otto Franklin	1	0	0	0	0	1	0
Otto II	10	0.035	0.19	0.114	9	0	1
Otto Sweetened	7	0.165	0.207	0.187	3	4	0
OTTOLLXOG	1	0	0	0	0	1	0
PALMER	1	0	0	0	0	1	0
PALMETTOHARMONY	1	0	0	0	0	1	0
PENNELL	3	0.083	0.099	0.091	0	3	0
Randy	1	0.201	0.201	0.201	1	0	0
Red Bardot	1	0	0	0	0	1	0
Red Kross	3	0.021	0.071	0.051	0	2	1
RELIEFNOW	1	0	0	0	0	1	0
Remission	1	0	0	0	0	1	0
SEQUATCHIESOUR	1	0	0	0	0	1	0
Shark Bait	1	0	0	0	0	1	0
Siskiyou Late & Carolina	1	0.078	0.078	0.078	0	1	0
Siskyou Gold	7	0.058	0.27	0.13	2	4	1
Sour G	1	0	0	0	0	0	1
Sour Space Candy	8	0.056	0.393	1	0	3	5
Sour Tsunami	1	0.03	0.03	0.03	0	0	1
Special Sauce	13	0.061	0.146	0.089	0	13	0
Spectrum	2	0	0	0	0	1	1
SSalpha	2	0.205	0.207	0.206	2	0	0
stormy	1	0	0	0	0	1	0
Stormy Daniels	2	0.046	0.053	0.049	0	0	2
Stout	15	0.039	0.141	0.082	6	7	2
Stray Cat	5	0.059	0.066	0.062	0	4	1
Suaver Haze	38	0.054	0.352	0.098	0	29	9
Sundance	1	0	0	0	0	0	1

Super CBD	9	0	0	0	0	7	2
Susie Q Baox	1	0.287	0.287	0.287	0	0	1
Susie-Q	1	0.122	0.122	0.122	0	0	1
Sweet	15	0.054	0.268	0.123	5	10	0
Sweet Carolina	1	0.063	0.063	0.063	0	0	1
Sweetened	132	0.064	0.443	0.144	26	96	10
Sweetened and Franklin	1	0	0	0	0	1	0
SWEETENED2	1	0	0	0	0	1	0
SWEETENEDOTTO	1	0	0	0	0	1	0
T1	42	0.053	0.433	0.142	0	26	16
T1&Berry Blossom	1	0.042	0.042	0.042	0	0	1
T2	1	0	0	0	0	1	0
Tangerine	1	0.055	0.055	0.055	0	0	1
The Haute Wife	1	0.248	0.248	0.248	0	0	1
The White CBG	1	1.879	1.879	1.879	0	0	1
The Wife	5	0.058	0.125	0.091	0	4	1
TN Select	1	0	0	0	0	1	0
TN-20	1	0.101	0.101	0.101	0	0	1
TREX	1	0	0	0	0	1	0
True Dream	1	0.113	0.113	0.113	0	0	1
Trump	2	0	0	0	0	2	0
Trump 1	11	0.047	0.186	0.092	7	2	2
Ultra Cherry	1	0.066	0.066	0.066	0	1	0
Very Berry	1	0	0	0	0	1	0
Virginia Hemp	3	0.072	0.072	0.072	0	1	2
Vonn	1	0.361	0.361	0.361	0	0	1
Watermelon	4	0	0	0	0	3	1
Watermelon Baox	1	0	0	0	0	1	0
Weedham	1	0.091	0.091	0.091	0	0	1
White 34	1	0.107	0.107	0.107	0	0	1
White CBG	4	0.032	0.067	0.045	0	0	4
White Grapefruit	2	0	0	0	0	0	2
Wife	10	0.077	0.301	0.142	0	5	5
WILDBERRYBLOSSOM	1	0	0	0	0	1	0
Wildhorse	1	0.103	0.103	0.103	1	0	0
WILLIAMS	1	0	0	0	0	1	0
Workhorse	1	0.149	0.149	0.149	1	0	0
Young Sim 10	14	0.065	0.258	0.14	1	11	2
Z1	1	0.203	0.203	0.203	1	0	0
Grand Total	**1107**						

Sample Year	Samples	Samples with no Variety	Samples with Variety
2018	307	164	143
2019	921	162	759
2020	205	0	205
Total:	**1433**	**326**	**1107**

ATTACHMENT E

TENNESSEE DEPARTMENT OF AGRICULTURE

Julius Johnson
COMMISSIONER

CONSUMER AND INDUSTRY SERVICES

Standard Practice for Field Sampling of Industrial Hemp Stands

Issued August 2015

1. Scope

1.1 This practice applies to field sampling of Industrial Hemp.

1.2 Samples collected under this procedure are acceptable for submitting to a qualified laboratory for Determination of Delta-9-Tetrahydrocannabinol (Δ^9THC) in Industrial Hemp.

1.3 The samples collected are intended to be representative of the THC content in the hemp stand growing in the cultivated growing area.

1.4 This practice describes the standard procedures for obtaining representative samples under the authority of Tennessee Code Annotated §43-27-101 et seq. and Tennessee Rule 0080-06-27.01 et seq.

1.5 *This practice does not purport to address all of the safety concerns, if any, associated with its use. The user of this standard shall exercise caution and follow applicable safety and health practices.*

2. Reference Documents

2.1 Tennessee Code Annotated §43-27-101 et seq.

2.2 Tennessee Rule 0080-06-27.01 et seq.

2.3 Health Canada – Industrial Hemp Technical Manual

2.4 United Nations Office on Drugs and Crime (UNODC) Recommended methods for the identification and analysis of cannabis and cannabis products.

2.5 Codex Alimentarus Recommended Methods of Sampling For The Determination Of Pesticide Residues For Compliance With MRLS CAC/GL 33-1999

3. Terminology

3.1 *Definitions:*

3.1.1 *sample* – the combined total number of hemp samples taken from the plants in the growing area..

3.1.2 *department* – the Tennessee Department of Agriculture.

3.1.3 *growing area* - -the land area on which industrial hemp is grown.

3.1.4 *licensee* – an applicant that has been approved to grow industrial hemp under 2014 Public Acts, c. 916, §5 and Tennessee Rule 0080-06-27.01 et seq.

3.1.5 *sample specimen* – the inflorescence of the hemp plant.

3.1.6 *Stand* - a group or growth of industrial hemp.

3.1.7 *THC* - Delta-9-Tetrahydrocannabinol (Δ^9THC*)*

4. Summary of Practice

4.1 This practice provides procedures for entering a growing area and collecting the minimum number of plant specimens necessary to represent a homogeneous composition of the stand that is to be sampled. An authorized representative of the Tennessee Department of Agriculture enters a growing area, strategically examines the growing area, establishes an approach for navigating the growing area and collects individual specimens of representative plants in order to obtain a representative sample of industrial hemp in the growing area. Because test methodology requires small aliquots of material, care must be taken to provide samples that accurately reflect entirety of the growing area.

4.2 Producers may not collect samples from their own growing facilities.

5. Interferences

5.1 The time of the growing season selected for collected the samples will impact the concentration of THC.

6. Equipment and Supplies

6.1 Garden pruners

6.2 Sample bags, paper.

6.2.1 The size of the bags will depend upon the number of clippings collected per growing area. Example Supplier: Tritechforensic http://tritechforensics.com/store/product/paper-evidence-bags-small-medium-large/

6.3 Security tape

6.4 Permanent markers

6.5 Sample collection forms

6.6 GPS Unit

6.7 Disposable gloves - Nitrile

7. Sampling Procedures

7.1 The Licensee or designated employee shall accompany the state inspector throughout the sampling process.

7.2 Surveillance of the growing area.

7.2.1 Inspector shall verify the GPS coordinates of the growing area as compared with the GPS coordinates submitted by the licensee.

7.2.2 The inspector shall estimate the average height, appearance, approximate density, condition of the plants, and degree of maturity of the inflorescences.

7.2.3 The inspector shall visually establish the homogeneity of the stand to establish that the growing area is of like variety.

7.2.3.1 Any concerns of stand homogeneity shall be immediately reported to supervisory staff.

7.3 Time of Sampling.

7.3.1 Unless otherwise instructed, routine sampling shall be conducted as the seed begins to mature, i.e. when the first seeds of 50% of the plants are resistant to compression. If Hemp crop is to be harvested earlier than above, TDA will need to be notified 2 weeks prior to planned harvest.

7.4 For purposes of determining the number of individual plant to select for sampling, one (1) acre of growing area shall be considered one (1) lot.

7.5 For growing areas of four (4) acres or less, select a minimum of four (4) plants for cuttings to form a composite sample.

7.6 For growing areas larger than ten (10) acres, the number of plants that will be selected to form a composite sample is based upon the Codex Alimentarius Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLS *CAC/GL 33-1999.*

7.6.1 The sample size, *n*, shall be calculated as follows:

$$n = \frac{n_0}{1 + (n_0 - 1)/N} = \frac{299}{1 + (298/N)}$$

where *n = minimum number of primary plants to be selected for forming a composite sample*

n_0 = 299 (95% probability to detect non-compliant sample with 1% chance of non-compliant incident (1 out of 100 plants possibly non-compliant))

N = Number of lots (acres of cultivation)

7.6.1.1 Example Calculations:

A 100 acre field: calculate 299/(1+(298/100)) = 299/(1+2.98)=299/3.98=75 plants -- total clippings 75x2 (one for each bag) – total 150 clippings

A 75 acre field: calculate 299/(1+(298/75)) = 299/(1+3.97) = 299/4.97 = 60 plants – total clippings 60x2 (one for each bag) – total 120 clippings

A 45 acre field: calculate 299/(1+(298/45)) = 299/(1+6.62) = 299/7.62 = 40 plants – total clippings 40x2 (one for each bag) – total 80 clippings

7.6.1.2 Total samples to be taken per number of acres

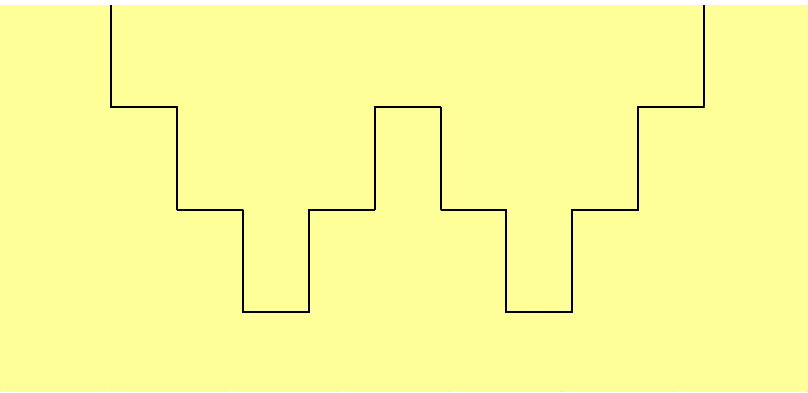
- Growing Area, acres (lots)	***n* – *number of plants***	**Retain Sample Multiplier**	**Total Clippings**
1	*4*	2	**8**
2	*4*	2	**8**
3	*4*	2	**8**

4	*4*	2	**8**
5	*5*	2	**10**
6	*6*	2	**12**
7	*7*	2	**14**
8	*8*	2	**16**
9	*9*	2	**18**
10	*10*	2	**20**
15	*15*	2	**30**
20	*18*	2	**36**
25	*24*	2	**48**
30	*28*	2	**56**
35	*32*	2	**64**
40	*36*	2	**72**
45	*40*	2	**80**
50	*43*	2	**86**
75	*60*	2	**120**
100	*75*	2	**150**
160	*104*	2	**204**

n is multiplied by 2 in order to split the total volume into test and retain containers

7.7 Collecting Samples from each Growing Area:

7.7.1 Always walk at right angles to the rows of plants. The tester shall always walk toward two points opposite each other. See example below.



7.7.2 While walking through the growing area, the inspector shall cut at least *n x 2* inflorescences at random but convenient distances. Avoid collecting sample specimens from the borders of the field.

7.7.3 The cut shall be made five to eight inches in length from the main stem that includes the leaves and flowers, the terminal bud that occurs and the end of the stem, or central cola cut that could develop

into a bud.

7.7.4 Utilize two sample bags for collecting sample cuttings, arbitrarily dividing the total volume equally into two sacks. Ensure that each sack has the minimum number of cuttings, *n*, as calculated by 7.6.1.

7.7.5 Seal each sack and record the sample number. Identify both sample sacks as being the same "sample #". The samples shall be identified using the following system for assigning sample numbering:

The sample number consists of:

Inspector Badge number (ID XXX), Hemp license number (IH XXXX), field number (same as that on sample inspection form), Fiscal Year (2016), sample number (starts with one and increases sequentially by one for each double sample for FY 2016.

Both Sample bag labels must have the sample number.

Once the sample is received by TDA lab personnel, one of the Sample bags will be selected for analysis and labeled "Test Specimen". The other sample will be marked "Retain Specimen" and kept in a secured place.

Example of sample Number: 123 1234 01 2016 001

Inspector Badge No.	Hemp Lic No.	Field No.	FY No.	Sequential No.
123	1234	01	2016	001

Record as: 123 1234 01 2016 001

7.7.6 Complete Industrial Hemp Sample Collection Form. Review form with the responsible party. Obtain signatures of responsible party and sign document. **Begin custody transfer documents.**

7.7.7 Samples must be stored in an area that is locked and secure.

7.7.8 Samples are routed to Tennessee Department of Agriculture, Consumer & Industry Services Division, Technical Services Laboratory.

ATTACHMENT F

Hemp Program Certification

by Charlie Hatcher, DVM, Commissioner of Agriculture

Pursuant to 7 C.F.R. § 990, I certify that the Tennessee Department of Agriculture has the resources and personnel necessary to carry out each of the practices and procedures identified in this plan.

Date: 10/12/2021

Respectfully,

Charlie Hatcher, DVM, Commissioner