West Virginia Department of Agriculture
Proposed Industrial Hemp Plan

West Virginia Industrial Hemp Development Act
West Virginia Code §§19-12E-1 *et seq*.

West Virginia Legislative Rule for Industrial Hemp
West Virginia Code of State Rules 61-29

Date of Resubmission: March 24, 2020

Proposed Effective Date: October 31, 2020

Overview of program

In 2002, the West Virginia Legislature passed the Industrial Hemp Development Act, which created the framework for legalized industrial hemp in West Virginia and paved the way for the growth and cultivation of industrial hemp in the State. Following passage of the 2014 Farm Bill, which authorized states to establish pilot programs for industrial hemp research, the West Virginia Department of Agriculture (WVDA) acted swiftly to implement the pilot program, based on the authority already granted in state law.

Since the inception of this pilot program, interest and investment in industrial hemp in West Virginia has grown exponentially. From 2017 to 2019, the number of license-holders increased from 46 to 165, and the acreage registered with the program recorded a corresponding increase, quadrupling to 641 planted acres over the same time frame. For the 2020 growing season, WVDA received 456 applications to grow and/or process hemp.

WVDA's current regulations and practices under its pilot program are very similar to those required by the Interim Final Rule. This means that West Virginia is well positioned to make a smooth transition into operating under a USDA-approved hemp plan. This document is an outline of the West Virginia Department of Agriculture's Industrial Hemp Program and is WVDA's formal submission to the USDA for review and approval.

Application process

WVDA will designate a time period for interested parties to apply for an industrial hemp license. Licenses will become effective, upon approval of the submitted application, for the calendar year for which the application is made and will expire on December 31 of that year. To participate in the WVDA's industrial hemp program, the applicant must complete the most current WVDA hemp application, submit geospatial coordinates and other required information concerning land where hemp operations will be performed, provide state and federal background checks and both an application and licensing fee.

Compliance Statement

By signing and submitting his or her industrial hemp application, each applicant understands and acknowledges that he or she is being licensed to grow hemp under the limited authority granted by the USDA Interim Final Rule on Hemp, the West Virginia Industrial Hemp Development Act and the WVDA through its Hemp Program. The applicant acknowledges and agrees that the actions of all individuals employed by or contracting with the applicant, and that the operations on all sites registered to the licensee are the responsibility of the applicant and can be imputed to the applicant for purposes of regulation by WVDA. Furthermore, the applicant acknowledges and agrees to the following terms and conditions:

- Any information provided to WVDA, except criminal history records provided to WVDA, may be publicly disclosed and be provided to law enforcement agencies without further notice to the applicant;
- The applicant agrees to allow any inspection and sampling that WVDA considers necessary;
- The applicant agrees to pay for any sampling and analysis costs that WVDA considers necessary;
- The applicant agrees to submit all required reports by the applicable due-dates specified by the Commissioner; and
- The applicant agrees to update information with WVDA as changes occur to the information submitted on applications.

Validation & Review of Applications

WVDA will review each application to ensure that all requested information has been provided. If the application is deemed incomplete, the applicant will be given an opportunity to provide the missing information before the application is rejected. Any applicant convicted of a felony for a controlled substance within the past 10 years will be ineligible for licensing. Any land owned by an individual with a felony conviction for a controlled substance within the past 10 years may be ineligible for inclusion on a license. Any other felonies of which an applicant has been convicted within the preceding 10 years will also be reviewed and may result in ineligibility.

License Process

All applicants who submit complete applications and otherwise satisfy all the requirements of the program will be issued a license. The license will be specific to, and will list, locations where the licensee is permitted to grow, process, cultivate, store, or handle raw industrial hemp. Applicants are responsible for both themselves and all individuals involved with planting, maintenance, harvesting, and processing of the industrial hemp grown under their license.

Information Maintenance & Reporting

WVDA maintains files for each licensee that contain contact information, application, license, geospatial location, legal description of land, sampling invoices, sampling chain of custody, THC results, all fees collected, key participants' names and titles, state and federal background checks for applicants (including key participants, where applicable). All records are kept on file for at least 5 years. While records are kept confidential in the ordinary course of operations, some or all of those records may be subject to the state's Freedom of Information Act, W. Va. Code §§29B-1-1- *et seq*., and subject to disclosure upon request.

WVDA will provide USDA with contact information for each licensee covered under the plan, including: name, address, telephone number, and email address. If the licensee is a business

entity, WVDA will provide USDA with the full name of the business, address of the principal business location, full name and title of the key participants, an email address, and the business entity's FEIN number. WVDA will also provide USDA with a legal description for all land licensed for the production, storage, growing, processing, or handling of raw industrial hemp, including a geospatial location for each site. WVDA will further provide USDA updates on the status of each license, including any status changes. All information reported to USDA will be submitted in accordance with its reporting requirements. WVDA will also report hemp crop acreage to the USDA Farm Service Agency.

Site Monitoring

Site inspections will be performed the same time pre-harvest samples are collected for THC compliance testing. Authorized WVDA representatives will perform site inspections to ensure compliance with State and Federal rules for hemp production in West Virginia. For the 2018 and 2019 growing seasons, WVDA successfully sampled and tested licensed locations within West Virginia, and will continue to do so. Site inspections may also be performed for other purposes, including research, education, collection of information, and compliance verification. WVDA intends to partner with USDA agencies, land grant institutions, and others to collect production and agronomic data for developing soil amendment, pest control, and recommendations for conservation best-management practices.

Field Sampling

Each licensee must contact WVDA at least 30 days prior to harvest of their licensed crop to schedule a time for sample collection. This timeframe allows adequate time to arrange for sample collection and delivery to the WVDA or equivalently accredited USDA approved laboratory for testing. Each growing area and variety (lot) will be sampled and tested to ensure that the post-decarboxylated tetrahydrocannabinol concentration level ("total THC") does not exceed 0.3%, plus or minus the measurement of uncertainty. Harvesting cannot occur until a WVDA representative has inspected and sampled that specific variety and/or location. Samples of hemp plant material from one growing area or variety (lot) shall not be commingled with hemp plant material from other lots. Each licensee is responsible for the costs associated with sampling and testing. Once a lot has been tested by WVDA and found to be compliant, it can enter the market.

During sampling, WVDA will collect hemp flower material from each variety or location (lot). The licensee or a designated employee shall accompany the WVDA representative throughout the sampling process. The WVDA representative shall verify the GPS coordinates of the growing area and compare them with the ones submitted by the licensee to ensure that the location was properly registered. The WVDA representative shall estimate the average height, appearance, density, plant condition, and degree of maturity of the inflorescences—or flowering buds. The WVDA representative shall visually establish the homogeneity of the stand to confirm that the growing area is of like variety. The sample size must be of adequate volume to accommodate laboratory tests and to ensure that the sample is representative of the lot. The WVDA

representative will utilize a paper bag and a hand pruner for collecting sample cuttings, securely seal each bag, and record the sample number. The WVDA representative will fill out a chain of custody form and sampling invoice for hemp samples taken at each location.

Lab Testing

The WVDA laboratory is one of ten labs in the country designated with a Level 1 status from the USDA Food Emergency Response Network. The WVDA laboratory scientists are internationally recognized for their research and method development activities. All WVDA READ Laboratories follow ISO Quality System Management Procedures, Technical Standard Operating Procedures, and Quality Manuals, and maintain a consultant pharmacist regarding all matters related to schedule 1 drugs used for controls. WVDA currently has seventeen methods that are ISO17025:205 accredited, and has further applied for its method of measuring total THC to be likewise accredited. American Association for Laboratory Accreditation (A2LA) plans to perform the laboratory audit in January 2020 for this method. The WVDA laboratory is a DEA schedule 1 laboratory.

The WVDA laboratory follows sample preparation procedures to ensure that the entire sample received by the laboratory is adequately prepared and homogenized prior to analysis (*see* Attachment 1). Once the sample is received and adequately dried, it is passed through a sieve to remove large non-grindable materials before being ground into a fine powder (*see* Attachment 2). Analysis of the homogenized sample's total THC is conducted using an Ultra-High-Performance Liquid Chromatography machine to chromatically separate and quantitate both Δ9-THC and THC-A. The formula [%total THC = % Δ9-THC + (%Δ9-THCA × 0.877)] accounts for the loss of carbon dioxide from the THC-A that results from decarboxylation (*see* Attachment 3). All samples are initially tested with several controls per batch to ensure that results are precise and accurate. If the calculated results are above 0.3%, taking into account the measurement of uncertainty, the samples are retested for confirmation.

WVDA follows a strict procedure on the calculation of measurement uncertainty for THC analysis in hemp. WVDA's method is based on the A2LA Guides for Estimation of Measurement in Testing. Reference materials are used to calculate the standard deviation. The expanded measurement of uncertainty is calculated at a 95% confidence level (*see* Attachment 4). The calculated measurement of uncertainty is indicated on each final report in a +/- format. The acceptable hemp THC level is based on the application of the measurement of uncertainty to the reported total THC content concentration level on a dry-weight basis, producing a distribution of 0.3% or less.

Violations and Enforcement

If a sample is determined to be above the acceptable limit of total THC (0.3% +/- MU), the licensee will be notified by certified mail, the contents of which will direct the embargo of the lot in violation, until such time destruction of the crop can be completed. Methods of crop destruction are based on scale, available equipment, and effectively rendering the crop irrecoverable. All crop

destruction is witnessed by WVDA employees, and report forms for all non-compliant plants will be submitted to the USDA.

WVDA will conclude that a licensee has negligently violated the state plan if it fails to provide a legal description of the land on which the licensee produces hemp or conducts hemp operations; fails to obtain a license or other required authorization from the West Virginia Department of Agriculture; or produces cannabis containing more than the acceptable level of total THC stated in the USDA Interim Final Rule.

A licensee will be required to comply with requirements established by WVDA to correct any negligent violation, including: (1) a reasonable date by which the licensee shall correct the negligent violation; (2) any requirement stipulating that the licensee shall periodically report to WVDA the licensee's compliance with the state plan for at least two calendar years from the date of the negligent violation (depending on the Commissioner's discretion); and (3) any other requirement deemed appropriate to address the negligent violation. A licensee who negligently violates the rules of the WVDA industrial hemp plan three times within a five-year period will be ruled ineligible to produce hemp in West Virginia for a period of five years beginning on the date of the third violation. WVDA shall conduct inspections to determine the corrective action plan or any other stated requirements have been implemented by the licensee.

If WVDA determines that a licensee has intentionally violated the provisions of its industrial hemp plan, the violation and licensee will be reported to the USDA, West Virginia Attorney General, the sheriff of the county in which the violation occurred, and the local detachment of the West Virginia State Police. Cannabis containing more than the acceptable level of total THC stated in the USDA Interim Final Rule, currently 0.5% total THC will be considered a negligent act on the part of the licensee, and other actions may be considered negligent or intentional based on additional factors, such as the licensee's state of mind.

Appeal Process

Once a licensee has been notified of a violation based on a non-compliant crop or other issue, he or she has the right to appeal the determination within ten (10) days of the date received. The licensee may request to schedule a hearing, during which time evidence and arguments may be presented. The appeal process is guided by the West Virginia Administrative Procedures Act, W. Va. Code §§29A-5-1 *et seq*., and the issues, once argued and briefed, are decided by the WVDA Commissioner.

Program Financing

WVDA operates as an interdisciplinary department, and many employees are cross-trained to assist with various programs; the industrial hemp program is no exception. Aside from WVDA's

full-time Hemp Program Coordinator, the following employees and divisions contribute to the operation and management of the WVDA Industrial Hemp Program:

- Executive Division, including the Commissioner, Deputy Commissioner, Chief of Staff, and General Counsel;
- Plant Industries Division, including plant regulatory officers;
- Regulatory and Environmental Affairs Division, including chemists, research chemists, hemp chemists, compliance officers, laboratory technical leaders, laboratory quality managers, and others;
- Business Development Division;
- Communications Division;
- Information Technology Division; and
- Administrative Services Division.

Due to the interdisciplinary structure of the Department, WVDA is confident that it has adequate personnel to properly implement this Plan. Further, the West Virginia Legislature has provided additional appropriations, both one-time and ongoing, to fund the program, thus indicating a commitment to support the industry.

Conclusion

In accordance with federal and state laws, WVDA does not discriminate in its programs and services on the basis of race, color, religion, sex, age, national origin or ancestry, disability (including blindness), medical condition, marital status, veteran status, or political affiliation.

WVDA retains the right to update this plan in accordance with West Virginia statues, legislative rules, and USDA guidelines.

Purpose

The purpose of this SOP is to provide general guidelines for the preservation, storage, and handling of *Cannabis sativa* samples.

Scope / Field of Application

This SOP is applicable to all *Cannabis sativa* samples collected for examination of THC.

Definitions and Acronyms

Sample receipt- The transfer of the sample from the Regulatory Officer to the WVDA READ staff.

Sample log-in- The inspection and check-in of the sample via paper and/or an electronic database in order to maintain the chain-of-custody.

Sample custody- A sample is under custody if:

a) It is in your possession, or
b) It is in your view, after being in your possession, or
c) It was in your possession and then you locked it up or placed it in a sealed container to prevent tampering, or
d) It is in a designated secure area.

Responsibilities

Laboratory personnel are responsible for applying proper techniques for receiving, preserving, storage, and handling of *Cannabis sativa* samples. These techniques are critical for maintaining the integrity of the samples.

Laboratory personnel are responsible for meeting with the Regulatory Officer upon sample delivery.

Laboratory personnel are responsible for receiving, logging in, and storing samples as outlined in this procedure. This responsibility also includes inspecting incoming samples for damage, missing seals, missing paperwork, or mismatch of paperwork to samples.

The Assistant Director, Quality Manager, or Technical Lead may resolve questions arising about the suitability of a particular sample for testing.

The Technical Lead is responsible for communication with customers when samples do not arrive in sufficient quantity or acceptable condition as determined by test methods or customer requirements.

Materials Required

Residue Laboratory Sample Log

Residue Custody Form
Computer capable of running the current version of USALIMS
Locked Freezers and cabinets for secure storage

Procedure

Sample Receiving Methods

Note: Samples are collected and delivered in person to the WVDA by Regulatory Officers. It is the Regulatory Officer's responsibility to ensure the sample has followed a proper transportation method.

1. **Sample Receiving Area**
   - 1.1 The sample receiving area is located in the designated upstairs office adjacent to the mailboxes in the WVDA READ building.
   - 1.2 Visitors entering the building with access to the sample receiving area must sign in at the front desk and be escorted behind the swipe card access door.
   - 1.3 Samples received outside of normal business hours must be coordinated with laboratory management.
   - 1.4 *Cannabis sativa* samples will be in custody at all times.

2. **Sample Receipt**
   - 2.1 The Regulatory Officer will hand deliver the *Cannabis sativa* samples since they cannot be mailed. Samples should arrive in an undamaged, paper bag and must be properly sealed. The Regulatory Officer will have the sample custody form that they began at the collection site.
   - 2.2 Only authorized staff members can signs the custody form for the samples and must document the receipt with the date, time, and their initials. A custody form is used for each sample. Any issues with the sample integrity should be resolved with the Regulatory Officer upon delivery. The staff member must note any damage or issue with the sample upon receipt on the custody form.
   - 2.3 If the staff member who signs for the sample is a different staff member than that of who logs-in the sample, the transfer to the log-in staff member must be documented on the custody form.

3. **Sample Receipt, Identification, and Log-in**
   - 3.1 Only authorized laboratory staff may log in the *Cannabis sativa* samples.
   - 3.2 Ensure that all paperwork is with the sample and that the sample seal matches the documentation.
     - 3.2.1 Samples may be rejected if there is any doubt that the integrity of the sample has been compromised or the sample has been improperly identified. Reasons for rejection may include, but are not limited to, the sample being too old, improper

packaging or handling, broken seal, paperwork that does not match the sample. If a sample is rejected, the technical lead, or authorized proxy, must consult with the Regulatory Officer. The reason for rejection must be documented. Any correspondence with the Regulatory Officer must be retained and kept with the sample forms.

3.2.2 When there is doubt as to the suitability of a sample for test, the sample should be held and stored in a way to maintain its integrity until guidance from the Regulatory Officer is received.

3.3 Samples will be logged into the USALIMS Pesticide Residue (PRL) database in order to maintain the chain-of-custody. Instructions for logging in sample data into the USALIMS Pesticide Residue module are outlined in the SOP *Pesticide Sample Entry into USALIMS*. Minimally the following information must be recorded: date / time / initials of receipt, if the sample is intact, delivery method, identification number, type of sample, analyte to be tested, and the date / time / initials that the sample was retrieved from the sample receiving area or receiver.

3.4 After logging in the first page (general tab) of the LIMS data for each sample, print corresponding labels with the identifying accession number. Accession numbers are generated in a non-repeating sequence by the LIMS software. One label should be placed onto the paperwork, one label should be placed onto the sample, and the remaining labels should be affixed to the corresponding paperwork and chain-of-custody form.

3.5 Samples shall be in custody at all times and stored in the manner they were received.

3.5.1 Laboratory staff are responsible for comparing the samples to the paperwork prior to initialing for the samples.

3.5.2 Initialing the chain of custody record transfers ownership and responsibility for the samples to the laboratory.

4. Ownership of Laboratory

4.1 Only authorized laboratory personnel may transfer the ownership of the sample from the receiving area to the laboratory.

4.2 Any manipulation of the sample must be documented on the sample custody log. This includes but is not limited to, the removal of a sample for testing purposes, shipment, or disposal.

4.3 When the original seal is broken after arriving to the laboratory, the laboratory should note who broke the seal. This may be documented by initial beside the seal number on the form.

4.4 The unique identifier, or accession number, must be carried through with the sample to its reporting stage. Stickers with this accession number should be printed from the WVDA LIMS and placed on all related laboratory items (tubes, bags, etc.) that need to be labeled with the identifying number. The identifying stickers should remain with the sample throughout its lifetime within the WVDA, regardless of the storage conditions.

5. Sample Storage and Preservation

Note: All samples must be stored under conditions that will preserve the sample integrity pending analysis, according to the following guidelines:

5.1 *Cannabis sativa* samples will be processed within 72 hours of receipt and will be stored at room temperature in the locked cabinet in the residue laboratory until prepared for analysis.

5.2 Samples that have been processed and cannot be analyzed the same day will be stored in a locked -20°C freezer.

5.3 Samples can become contaminated if they come in contact with hands, therefore, appropriate protective equipment should be worn at all times during sample manipulations for preservation.

5.4 Samples can become contaminated from materials and equipment used to manipulate and preserve the sample, therefore, all materials that come in direct contact with the sample should be kept clean.

5.5 Samples should be stored in areas that will minimize the likelihood of cross-contamination with controls.

5.6 Samples should be stored in a way that the packaging and/or seals are not damaged.

5.7 Any adverse environmental storage areas are avoided.

5.7.1 Samples should be protected from sunlight and humid conditions which may initiate degradation of sample components.

5.8 Delaying sample preservation may cause unwanted growth or chemical reactions to occur, altering the original sample composition.

5.8.1 Improper sample preservation may adversely affect analytical results.

5.9 Unauthorized access to the samples is restricted by a key card and locked cabinets during hours outside of the normal work day.

5.10 Inadequate sample volume may prohibit the appropriate analyses from being performed.

5.11 Samples that are received at room temperature may be stored in the locked cabinet in the residue laboratory until prepared for analysis.

5.11.1 Refrigerators and freezers that used for samples, sample homogenates and sample extracts are maintained at required temperatures. Temperatures are checked and recorded on a daily basis during the working days.

6. Sample Retention Time

6.1 *Cannabis sativa* samples with THC levels <u>below</u> one percent will be retained for a period of two weeks in a -20°C freezer and then all parts (leaves, stems, seed) will be disposed after steam sterilizing at 121°C for 15 minutes.

6.2 *Cannabis sativa* samples with THC levels <u>above</u> one percent will be retained in a -20°C freezer for the potential release to the WV State Police Custody. When a sample has a THC level above one percent, notify the assistant director immediately.

7. Sample Disposal

7.1 Disposal of *Cannabis sativa* samples will be documented on the Residue Chain of Custody form. The date that the sample is disposed of should be recorded.

7.2 *Cannabis sativa* samples with THC levels below one percent will be steamed sterilized at 121°C for 15 minutes and then disposed in the regular waste.

Documentation

The following Quality Records shall be generated and managed:

Required Record	Custodian
Residue Chain of Custody Form	Personnel who possess custody of a sample
Residue Laboratory Sample Log-In Form	Sample Receiving Area

Reference Procedures

WVDA.GEN.00060 – Pesticide Sample Entry into USALIMS
WVDA.QSP.4-4-1- Contract Review

Reference

EXT- 268 Industrial Hemp Technical Manual – Standard Operating Procedures for Sampling, Testing and Process Methodology, Health Canada, June 1, 2004.
EXT-269 Guidance for State Medical Cannabis Testing Programs, Association of Public Health Laboratories, 2016.
EXT-270 61CSR20 Title 61 Legislative Rule Department of Agriculture, Series 29, Industrial Hemp
EXT-5 ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories, Subclause 5.8

Appendix

NA

Document History

Q Pulse

Purpose

To instruct analysts on the homogenization of industrial Cannabis sativa ~~Hemp~~ samples which involve drying, sorting, and grinding of *Cannabis sativa* ~~Hemp~~ samples.

Scope / Field of Application

This procedure outlines the proper techniques for the sample preparation and homogenization of industrial *Cannabis sativa* ~~Hemp~~.

Definitions and Acronyms

1. Homogenous – having a uniform composition or structure.
2. Sieve – A utensil with a frame surrounding a mesh that is used to separate large particles from smaller ones.

Responsibilities

All of the analysts in the Residue Laboratory are responsible for performing this procedure. It is the responsibility of the analysts to inspect the equipment, and working areas for cleanliness both before and after the samples are prepared and to check that the correct labels are on the sample containers. Any non-compliance is reported to the Technical Lead or Laboratory Assistant Director.

References

EXT- 268 Industrial Hemp Technical Manual – Standard Operating Procedures for Sampling, Testing and Process Methodology, Health Canada, June 1, 2004.
EXT-269 Guidance for State Medical Cannabis Testing Programs, Association of Public Health Laboratories, 2016.

Safety Considerations

Always wear appropriate personal protective equipment; including lab coats, gloves, and eye protection.

Equipment and Materials Required

1. Aluminum Foil, Food Service or heavy weight
2. Sieve - #8 Mesh
3. Polypropylene specimen containers with caps
4. Analytical Mill, Tekmar A-10 or equivalent
5. Hand operated cutters or shears
6. Drying oven, 90°C
7. Large desiccator
8. Balance, Analytical

Procedure

1.0 Cannabis sativa ~~Hemp~~ Sample Drying

1.1 Form a tray with the foil by folding up the sides.

1.2 Spread small portions of the entire Cannabis sativa ~~Hemp~~ sample onto the folded tray. Record the weight of the foil tray, and then the foil tray with the sample on the Cannabis sativa ~~Hemp~~ Sample Preparation form.

1.3 Dry the sample at 90° ± 5°C for approximately 2 hours.

1.4 Record the oven temperature, and time in and out of the oven on the Cannabis sativa ~~Hemp~~ Sample Preparation form.

1.5 Allow the sample to cool at least 30 minutes in a desiccator.

1.6 Record the weight of the sample and foil on the Cannabis sativa ~~Hemp~~ Sample Preparation form.

1.7 Dry the sample for an additional 15 minutes at 90° ± 5°C, and then transfer to the desiccator for at least 30 minutes to cool. Record the weight of the sample and foil on the Cannabis sativa ~~Hemp~~ Sample Preparation form. Repeat this step until a constant weight is achieved.

1.8 The remaining sample can be dried to the desired moisture content using the calculations from the Moisture in Cannabis sativa ~~Hemp~~ Worksheet.

2.0 Cannabis sativa ~~Hemp~~ Sample Sieving

2.1 Stems and leaves are sorted from the Cannabis sativa ~~Hemp~~ prior to homogenization.

2.2 After the Cannabis sativa ~~Hemp~~ sample is cool, gently work the entire dried sample through the #8 mesh screen sieve.

2.3 Place the sieved sample back onto the foil tray including any leaves or flower pieces that were too large to work through the mesh.

2.4 The seeds and stems are saved for disposal after sterilization.

3.0 Cannabis sativa ~~Hemp~~ Sample Homogenization

3.1 Place the sample into the grinding chamber of the analytical mill and grind for approximately 30 seconds, or until a fine texture is achieved.

3.2 Using the foil tray to catch any spilled sample, pour the sample from the analytical mill into the pre-labeled specimen cup.

3.3 Repeat steps 3.1 and 3.2 until the entire sample has been ground.

3.0 Records

3.1 Document that the sample has been prepared by initialing and dating the Residue Sample Logbook in the prep column on the line corresponding to the specific sample accession number.

3.2 The Cannabis sativa ~~Hemp~~ Sample Preparation form should be completed and maintained for each batch of samples dried in an oven.

3.3 Records will be maintained for inspections, reviews, audits or assessments.

4.0 Sample Storage, Disposal and Clean-up

4.1 Unprocessed samples (seals intact) shall be stored at room temperature in a locked cabinet in the Pesticide Residue Laboratory.

4.2 Processed samples that cannot be analyzed the same day will be stored in a locked -20°C freezer.

4.3 Violative samples shall be retained and investigators notified for immediate pickup.

4.4 Non-violative samples may be retained for method development work or disposed after steam sterilizing at 121°C for 15 minutes.

Documentation

The following Quality Records shall be generated and managed:

Required Record	Custodian
Residue Sample Logbook	Analyst
Cannabis sativa ~~Hemp~~ Sample Preparation form	Analyst

Appendix

Not applicable

Document History

Q Pulse

Purpose

The purpose of this procedure is to describe the sample preparation and quantitative analysis of cannabinoids in cannabis sativa samples by UHPLC using PDA detector.

Scope / Field of Application

This method is applicable to all cannabis sativa samples that are being analyzing for Tetrahydrocannabinolic acid (THC-A) and Tetrahydrocannabinol (THC)

Responsibilities

All analysts in the Agricultural Pesticide Residue Laboratory are responsible for performing this procedure, documenting all appropriate quality control, and reporting the results to the compliance officer.

References

Adapted from Reuter, Wilhad M. (2017) *Analysis of Cannabinoids in Cannabis by UHPLC Using PDA Detection.* Published by PerkinElmer

Suitable Matrix and Specimens

This method is suitable to the determination the chromatographic separation and quantitative monitoring of cannabinoids sativa, including THC and THC-A.

Safety Considerations

When working with samples always wear a laboratory coat, eye protection, and disposable nitrile gloves.

Equipment and Materials Required

Materials:
Column: Restek Force C18 3µm, 150x3.0mm (Cat# 963436E) or similar
Acetonitrile: VWR LC/MS grade (Cas# 75-05-8) or similar
Methanol: Honeywell LC/MS grade (Product# 34966) or similar
Water: Alfa Aesar Ultrapure HPLC (Cat# 22934) or similar
Formic Acid: Thermo (Cat# A117) or similar
50mL tubes: Thermo (cat# 339653) or similar
15mL tubes: Thermo (cat# 339651) or similar
Glass Pasteur pipettes: Wheaton (cat# W357331) or similar
0.45µm filters or filter vials
Standards:
THC: Cerilliant 1mg/mL in MeOH (Cat# T-005) or similar
THC-A: Cerilliant 1mg/mL in Acetonitrile (Cat# T-093) or similar
Equipment:

Analytical balance: Sartorius analytic, or similar
Vortex Mixer
UHPLC with Atlus A-30 PDA Detector– PerkinElmer
Pipettes:
Eppendorf 10µL
Eppendorf 20µL
Eppendorf 100µL
Eppendorf 200µL
Eppendorf 1mL
Eppendorf 10mL

Conditions for Acceptance of Samples

Samples must have a custody seal intact and signed by an inspector. The card must be legible and filled out correctly.

Specimen Handling

Samples are received in the sample receiving area. Sample receiving, storage, preservation, and handling SOP for Cannabis is followed. After a sample has been analyzed, the reserve samples are stored in locked freezer for secure storage.

Conditions for Sample Preparation Required

Follow sample preparation in SOP Cannabis sativa Sample Preparation for THC Analysis.

Controls

A past proficiency testing sample (PT) is run as a test control with each set of samples. The result of the test control is compared from several laboratories to determine the testing performance.

Checks and Calibrations

All prepared solutions are recorded in the Solution Preparation Log. Balances are calibrated annually in accordance with ISO 17025. An in-house calibration is performed monthly and two weights are checked daily and recorded.

Analysis and Interpretation of Data and Test Validity

The control sample must be compared to the other laboratories

Competency and Proficiency

All analysts have received training on the HPLC, including basic HPLC, troubleshooting, method development, and developing stability indicating methods

Procedure

Extraction:

Generated from Reuter, Wilhad M. (2017) *Analysis of Cannabinoids in Cannabis by UHPLC Using PDA Detection.* Published by PerkinElmer

1. Weigh one gram of ground-up dried hemp sample in duplicate into a 50 ml tube and record weight to nearest g
2. Add 10mL of methanol then vortex for three minutes
3. Filter 2 ml of the supernatant into a 15 ml tube though 0.45-µm filters
4. Dilute the filtered supernatant 3-fold with methanol (note: this resulted in overall 30-fold concentration dilution with respect to the initial product)
5. In a HPLC vial, dilute extract using 10µL extract in 990µL of diluent
6. Analyze by UHPLC, begin analysis same day and/or refrigerated until further use

Working Standard Preparation:

1. Add the entire 1 mL (100-µg/mL) of each standard to a 25-mL volumetric flask
2. Fill to mark with the 80:20 methanol/water diluent
    - This creates a solution with 500ppm THC/THC-A
3. Use serial dilutions of the previous stock to create a level-6 calibration standard curve, 50, 20, 5, 1 and 0.5-µg/mL

Experimental:

Hardware/Software: A PerkinElmer Flexar™ HPLC System was used, including a quaternary pump, autosampler with Peltier cooling, column heater and PDA (photodiode array) detector. A PerkinElmer Brownlee™ SPP C18, 2.7 µm, 3.0 x 150mm column was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control, data analysis/processing was performed via the PerkinElmer Empower 3 CDS software.

Method parameters:

The LC method parameters are shown in Table 1.

Table 1. HPLC Method Parameters.

Column:	Restek Force C18 3µm, 150x3.0mm (Cat# 963436E) or similar
Mobile Phase:	Solvent A: water with 0.1% formic acid Solvent B: acetonitrile with 0.1% formic acid Solvent program:

Step	Step Time (min.)	Flow Rate (mL/min.)	%A	%B

	0 (Equil)	4.5	1.0	30.0	70.0
	1	4.0	1.0	5.0	95.0
	2	2.0	1.0	5.0	95.0

Analysis Time:	6.0 min.; equilibration time: 4.5 min.
Flow Rate:	1.0 mL/min.
Pressure:	4600 psi/317 bar maximum
Oven Temp.:	40 °C
PDA Detection:	Wavelength: 228 nm
Injection Volume:	10 µL
Sampling (Data) Rate:	10 pts./sec
Diluent:	80:20 methanol/water

Calculations

1. Analyze data using Empower 3 software***.
2. Get the amounts of THC and THC-A to calculate the Total THC in the sample
   - The amount (%) of THC-A and THC are calculated using the recovery efficiency and sample weight added originally to give a weight percent of each analyte using the formula below where X = recovered ppb of THC or THC-A, Y = % recovery, Z = sample weight in mg
     - (X*(300/1000) *(100/Y)) / Z
   - Total THC is %THC + %THC-A x 0.877

Documentation

The following Quality Records shall be generated and managed:

Required Record	Custodian
Empower 3 Software THC Report	Analyst

Appendix

N/A

Document History

Q Pulse

Prepared by: Brenda Keavey Date: 2015/03/17

Reviewed by: Zachary Kuhl Date: 2015/03/19

Approved by: Brenda Keavey Date: 2015/03/19

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

Purpose

This procedure describes the process for the estimation of measurement uncertainty in a test method.

Scope / Field of Application

All measurements from chemical and microbiological test methods, except when the test methods preclude such rigorous calculations that are taken in collecting data for reporting to the customer.

Note – It is important to understand the major factors of uncertainty and provide appropriate control for all such factors. Concurrent analysis of reference materials or control samples with the test portion can be performed in place of purely mathematical estimation of uncertainty. If possible, the reference material or control sample shall be of identical or similar matrix as the matrices routinely tested by the test method. The uncertainty of the method can be estimated for the class of matrix and the variation described as the uncertainty in testing the specific matrix class at the average amount of analyte detected.

Definitions and Acronyms

Coverage factor – The number that is multiplied by the standard uncertainty to produce an expanded uncertainty (U) estimate that will contain a large fraction of all values that might be obtained on a test. The coverage factor is commonly noted as k; k=2 is used for 95% coverage, and k=3 for 99% coverage.

Combined standard uncertainty (U_c) – The result of the combination of the standard uncertainty components.

Expanded uncertainty (U) – The uncertainty obtained by multiplying the combined standard uncertainty by the coverage factor, k.

Measurement uncertainty – The parameter associated with the result of a measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurand.
Measurand – The specific quantity being measured (i.e. the concentration of the analyte).
Repeatability conditions – Identical samples prepared at the same time, by the same analyst, under identical conditions, run on the same instruments are repeatability conditions.
Reproducibility conditions – Reproducibility conditions are the identical samples analyzed under different conditions, including the following: different times, different analysts, or different laboratories.
$\mathbf{S_r}$ – The standard deviation of results produced under repeatability conditions.
$^{S}\mathbf{R}$ – The standard deviation of results produced under reproducibility conditions.
Standard deviation, S – The positive square root of the variance, calculated as:

$$\text{Standard deviation, } S = \sqrt{\frac{\sum (X_i - X_m)^2}{n - 1}}$$

Where, X_i is an individual value
X_m is the mean value
n is the number of measurements

Standard uncertainty – The estimated standard deviation.
t-statistic – A family of distributions indexed by its degrees of freedom. The *t*-models are unimodal, symmetric, and bell-shaped, but generally have fatter tails and a narrower contour than the Normal model. As the degrees of the freedom increase, *t*-distributions approach the Normal model. *t*-statistic values are commonly found in statistics reference books, or can be calculated with statistics or some spreadsheet software.
Type A Uncertainty – Determined through calculation from a series of repeated observations using statistical methods. Type A uncertainty assumes a normal Gaussian distribution.

Type B Uncertainty – Determined through judgment, based on data in calibration certificates, previous measurement data, experience with the behavior of the instruments, manufacturer's specifications, and all other relevant information. Type B is estimated by other means than Type A and is limited to rectangular distribution.

Responsibilities

The Technical Leads are responsible and have authority for the identification of measurement uncertainty category for each test within the accreditation scope, the collection of data and the calculation of the estimated uncertainty for each analytical method and analyte within their respective labs. They are also responsible for forwarding the completed Measurement Uncertainty forms to the Assistant Director for review and to the Quality Manager to file.

The Quality Manager is responsible for maintaining a copy of the completed Measurement Uncertainty worksheets.

Materials Required

Electronic spreadsheet capabilities

Procedure

1.0 Specification

Write down a clear statement of what is being measured, including the relationship between the measurand and the parameters (e.g., measured quantities, constants, standards, etc.) upon which it depends. Where possible, include corrections for known systematic effects. The specification information, if it exists, is normally given in the relevant standard operating procedure (SOP) or other method. Data can be reported as "x $\pm$ μ". This procedure explains several ways of determining "μ" for a test method. Most of WVDA's test methods will be either qualitative (Category I) or quantitative methods (Category III). Methods in Category I do not require that μ be estimated. Methods in Category III can estimate μ by using the standard deviation of the test method obtained from the process control chart. The standard deviations should be multiplied by 2 to give the μ corresponding to the 95% confidence level. The uncertainty values calculated using this procedure must be kept on file with the QM. Unless specified by the customer, the estimation of measurement uncertainty is not documented on the final report. A more rigorous description of the estimation of uncertainty process is provided below.

2.0 Identify Uncertainty Sources

List the possible sources of uncertainty. This will include sources that contribute to the uncertainty on the parameters in the relationship specified in 1.0, but may include other sources and must include sources arising from chemical assumptions. The Technical Leads identify the measurement uncertainty category (I-V listed below) for the tests identified on the laboratory's scope of accreditation. This information is recorded on the Measurement of Uncertainty worksheet.

2.1 Category I

This category is for qualitative or semi-quantitative tests for which measurement uncertainty estimation is not required. (Methods that report on a qualitative scale that are based on an underlying continuous quantitative scale fall under category II or III; for example, those reporting presence/absence based on a qualified number.) These test methods are listed and an uncertainty value of "not required" is entered in the value column on the measurement uncertainty worksheet.

2.2 Category II

In cases where a well-recognized test method (standard method) specifies limits to the values of the major sources of uncertainty of measurement and specifies the form of presentation of calculated results, the laboratory reports the measurement uncertainty stated in the test method. This is done only if the science of the test method is followed and results are reported as required in the test method. The estimated measurement uncertainty values adopted for use are recorded in the "value" column of the measurement uncertainty worksheet.

2.3 Category III

Standard test methods such as chemical, environmental, or biological test methods based on published regulatory or consensus methods (examples: FDA, EPA, AOAC, ASTM, APAH/AWWA) for which the measurement uncertainty is not defined in the method. The laboratory must determine the measurement uncertainty as described later in this procedure. The estimated measurement uncertainty values determined for use are recorded in the "value" column on the measurement uncertainty worksheet.

2.4 Category IV

Test methods that need identification of the major components of uncertainty and a reasonable estimate of measurement uncertainty. The laboratory must determine the measurement uncertainty as described later in this procedure. The adopted for use are recorded in the "value" column on the measurement uncertainty worksheet.

2.5 Category V

Test methods which require the identification of all components of uncertainty. Detailed measurement uncertainty budgets are calculated in accordance with published methods consistent with those described in the "ISO Guide to the Expression of Uncertainty in Measurement". This process is summarized later in this procedure. The estimated measurement uncertainty values adopted for use are recorded in the "value" column on the measurement uncertainty worksheet.

3.0 Estimating Uncertainty

Measure or estimate the size of the uncertainty component associated with each potential source of uncertainty identified. It is often possible to estimate or determine a single contribution to uncertainty associated with a number of separate sources. It is also important to consider whether available data accounts sufficiently for all sources of uncertainty, and plan additional experiments and studies carefully to ensure that all sources of uncertainty are adequately accounted for.

3.1 Using the Standard Deviation of the Test Method

This procedure applies to Category III test methods. The standard deviation of a test method may be used as the estimate of measurement uncertainty if the reproducibility

data is such that variability due to all of the major sources of uncertainty is included. Other sources of uncertainty may exist, so determine the standard deviation(s) as described here, and use it as the first entry in the budget of the Root Sum Square (RSS) method described in the next section. The A2LA Guides for Estimation of Measurement Uncertainty in Testing (G104) and Microbiological Counting Methods (G108) referenced in an earlier section may be referred to for additional specific information if further explanation is required.

a. Perform spiked determinations in a matrix using a Reference Material of certified or otherwise known value. The spike level should be at or near the level at which the analyte is typically found.
b. The measurement process should be the same as for normal samples, and should include, wherever possible, the entire process including sub-sampling and sample preparation where appropriate.
c. The measurement process should be shown to be in statistical control at the time of measurement. Determinations from out-of-control processes may not be used.
d. Calculate concentration and percent recovery.
e. Calculate the standard deviation, s.
f. Calculate the expanded measurement uncertainty at the 95% confidence level:

$$U = k*s$$

(for 95% and 20 data points, use k=2; for 95% and less than 20 data points, use the appropriate t statistic for k)

Documentation of this process and calculation is required. The Standard Deviation Form is used to record this information.

4.0 Root Sum Squared

This method applies to category IV and V test methods. In certain instances described above this method may apply to category III tests.

a. Clearly define what is being measured.
b. Identify every possible source of uncertainty by reviewing the method and entering it in the Uncertainty Budget Spreadsheet (electronic).
c. Decide whether the components of the sources are to be included when running laboratory control samples.
d. Quantify all the components and all values should be in the same units. This may be accomplished by converting everything into percentages (%), if necessary. Possible sources of quantitative estimates include method validation studies, information published from methods or textbooks, calibration certificates, manufacturer's specifications, or experience.

e. Combine the components by squaring all independent components, add them, and then take the square root of the sum. This is called the root sum square method and gives the combined standard uncertainty (U_c).

f. Expand the combined standard uncertainty. Multiply the U_c by a coverage factor based on the level of confidence needed. In most cases a confidence level of 95% is sufficient, so k=2. If 99% confidence is required, k=3.
When estimates are based on limited data, k may vary according to the student t distribution.

5.0 Reporting Uncertainty Measurements

The extent of reporting of the estimates of uncertainty depends upon the needs of the customer, the specifications for the test, and the intended use of the results. If the customer does not desire the estimate, it is not necessary to report it. The estimate, though, is still calculated along with the means of making the estimate. Proper documentation is sufficient enough to replicate and confirm the calculation.

Reported uncertainties are to the same number of significant figures and in the same units as the result. The estimate should also state the level of confidence associated with the coverage factor.

Alternate Procedure

Concurrently analyze reference materials or control samples with test samples to estimate uncertainty. This is generally achieved during validation of methods.

Documentation

The following Quality Records shall be generated and managed:

Required Record	Custodian
Uncertainty of Measurement Worksheet	Technical Lead
Standard Deviation Worksheet	Technical Lead
Uncertainty Budget Spreadsheet (electronic)	Technical Lead

Reference Procedures

All test methods requiring uncertainty estimation.

References

ISO/IEC 17025 Subclause 5.4.6

A2LA; Policy on Estimating Measurement Uncertainty for Testing Laboratories (P103), ~~February 8, 2005,~~ December 6, 2013.
A2LA Guide for Estimation of Measurement Uncertainty in Testing (G104), ~~July 2002,~~ December 4, 2014.

Annex to the A2LA Policy of Measurement Uncertainty for Life Sciences Testing Laboratories, American Association for Laboratory Accreditation (P103b), ~~October 18, 2007~~ September 22, 2010.

A2LA; Guidelines for Estimating Uncertainty for Microbiological Counting Methods (G108), ~~November 5, 2007~~, September 3, 2014.

Eurachem / CITAC. Quantifying Uncertainty in Analytical Measurement (3rd Edition), 2012.

Appendix

N/A

Document History

Version #	Status (I, R, C)	Date Approved	Location of Change History	Name & Title	
				Author	Approving Official
1	I	2012/11/07		Amie Minor, Quality Manager/ Research Scientist	Reviewed by: Kellie Littlefield, Microbiologist I, Approved by: Brenda Keavey, Assistant Director
2	R	2012/11/27	Changes to header and footer made, addition of Document History section	Amie Minor, Quality Manager/ Research Scientist	Reviewed by: Kellie Littlefield, Microbiologist I, Approved by: Brenda Keavey, Assistant Director
2.1	R	2013/05/03	Minor spelling corrections	Amie Minor, Quality Manager/ Research Scientist	
3.0	R	2014/03/14	Changes throughout	Amie Minor, Quality Manager/ Research Scientist	Reviewed by: Zachary Kuhl, Microbiologist I, Approved by: Brenda Keavey, Assistant Director

3.1	R	2015/03/24	References Updated	Brenda Keavey, Assistant Director	Reviewed by: Zachary Kuhl, Microbiologist I; Approved by: Brenda Keavey, Assistant Director

Status= I-Initial, R-Revision, C-Cancel