



Washington
State Department of
Agriculture

Testing Protocol for Identifying Delta-9 Tetrahydrocannabinol (THC) Concentration in Hemp

This document is based on language from USDA's interim final rule establishing a U.S. Domestic Hemp Production Program, with adjustments to align to Washington State's RCW 34.05.353.

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AGR PUB 501-811 (N/12/19)

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Purpose

1. Standard testing procedures are specified for samples taken in accordance with the Sampling Procedures for the WSDA Hemp Program to measure the delta-9 tetrahydrocannabinol (THC) concentration levels of those samples on a dry weight basis. Hemp testing laboratories are not required to be ISO accredited, although WSDA strongly encourages adherence to the ISO 17025 standard.
2. The results are intended to measure the THC content of composite hemp samples collected from a designated “lot” of hemp crop acreage designated by a hemp producer. The purpose of the measurements are to determine whether the THC concentration of the tested material is within the acceptable hemp THC level.
3. As required under the WSDA hemp program regulation, laboratories conducting testing of hemp must conduct analytical testing for purposes of detecting the concentration levels of delta-9 tetrahydrocannabinol THC and shall meet the following standards:
 - (a) Laboratory quality assurance must ensure the validity and reliability of test results;
 - (b) Analytical method selection, validation, and verification must ensure that the testing method used is appropriate (fit for purpose) and that the laboratory can successfully perform the testing;
 - (c) The demonstration of testing validity must ensure consistent, accurate analytical performance; and
 - (d) Method performance specifications must ensure analytical tests are sufficiently sensitive for the purposes of the detectability requirements of this part.
 - (e) At a minimum, analytical testing of samples for delta-9 tetrahydrocannabinol concentration levels must use High-Performance Liquid Chromatography (HPLC) or (LC). The testing methodology must consider the potential conversion of delta-9 tetrahydrocannabinolic acid (THCA) in hemp into delta-9 tetrahydrocannabinol (THC) and the test result reflect the total available THC derived from the sum of the THC and THCA content.
 - (f) The total delta-9 tetrahydrocannabinol concentration level shall be determined and reported on a dry weight basis.
 - (g) Any sample test result showing with at least 95% confidence that the THC content of the sample is higher than the acceptable hemp THC level shall be conclusive evidence that the lot represented by the sample is not in compliance with this part.
4. Laboratories approved for THC testing must also be registered with DEA to handle controlled substances under the Controlled Substances Act (CSA), 21 CFR part 1301.13.

General Sample Preparation and Testing Procedures are as follows:

1. Laboratory receives sample.
 2. Dry sample to remove the majority of water.
 3. Mill and “manicure” sample through a wire screen no larger than 1.5 x 1.5mm to discard mature seeds and larger twigs and stems.
 4. Separate sample into a test and retain specimens.
 - a. Test specimen: go to step 5
 - b. Retain specimen: package and store until needed. When needed go to step 5.
 5. Determine moisture content or dry to a consistent weight (meeting criteria).
 6. Perform chemical analysis.
 7. Calculate total THC on a dry weight basis. Test results should be determined and reported on a dry weight basis.
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(A) Samples shall be received and prepared for testing in a WSDA approved, DEA registered laboratory as follows:

- (1) Once the composite sample is received by the laboratory, the laboratory shall dry all of the leaf and flower (not obvious stem and seeds) of the composite sample until brittle in a manner that maintains the THC level of sample. Samples are to be dried to a consistent loss (typically 5- 12% moisture content) so that the test can be performed on a dry weight basis, meaning the percentage of THC, by weight, in a cannabis sample, after excluding moisture from the sample. The moisture content is expressed as the ratio of the amount of moisture in the sample to the amount of dry solid in the sample.
- (2) The laboratory shall mill and manicure samples through a wire screen no larger than 1.5 x 1.5mm to discard mature seeds and larger twigs and stems.
- (3) The laboratory shall form sieve a “Test Specimen” and a “Retain Specimen.” One sample part shall be selected for analysis and labeled “Test Specimen”. The other sample part shall be marked “Retain Specimen” and shall be packaged and stored in a secured place.
- (4) The laboratory shall then determine moisture content or dry to a consistent weight.

- (5) The laboratory will then perform chemical analysis on the sample using High-Performance Liquid Chromatography (HPLC) or (LC) which is a scientific method (specifically, a type of chromatography) used in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material to separate and analyze compounds. Under the terms of this part, HPLC is one of the valid methods by which laboratories may test for THC concentration levels.
- (6) The laboratory will then calculate total THC on a dry weight basis.

References

E. Small and H. D. Beckstead. 1973. Common Cannabinoid Phenotypes in 350 stocks of Cannabis. J. of Natural Products. 36(2): 144-165.

United Nations Office on Drugs and Crime: *Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products*. ISBN 978-92-1-148242-3.

(B) Testing Methods The total available THC, derived from the sum of the THC and THCA content, shall be determined and reported on a dry weight basis.

Laboratories shall use appropriate, validated methods and procedures for all testing activities and evaluate measurement of uncertainty. Laboratories shall meet the AOAC International standard method performance requirements (SMPR) for selecting an appropriate method. The range of estimated uncertainty is reported as a \pm value and is the same unit as the hemp THC threshold (0.3% THC), following best practices for significant figures and rounding.

There are resources available for defining, guiding, and calculating measurement uncertainty.

They include the GUM, ISO, and Eurachem. It is necessary for the laboratory to determine the uncertainty of accuracy (u_{bias}), repeatability (u_r), and reproducibility (u_R) for each validated method. Once the expanded measurement uncertainty (U) is determined, then the confidence interval can be calculated around a designated threshold such as the hemp THC threshold (0.3% THC).

Based on the aforementioned resources, the following equation is recommended:

Equation:
$$U = k \times u_c$$

Where,

$$u_c = \sqrt{u_r^2 + u_R^2 + u_{bias}^2}$$

And:

u = standard uncertainty (standard deviation)

u_r = uncertainty due to repeatability

u_R = uncertainty due to reproducibility

u_{bias} = uncertainty due to accuracy (bias)

u_c = combined standard uncertainty

U = Expanded uncertainty = $\frac{u}{\text{Mean}} * k_{95\% \text{ confidence level}}, k = 2$

k = coverage factor, use 2 for a 95% confidence level

References

ISO 17025. General requirements for the complete testing and calibration laboratories.

Food and Drug Administration, Office of Regulatory Affairs, *ORA Laboratory Manual Volume III Section 4*, Basic Statistics and Data Presentation (current version).

AOAC Standard Method Performance Requirements (draft) AOAC SMPR 2019.XXX; Title: Quantitation of cannabinoids in plant materials of hemp (low THC varieties 4 Cannabis spp.).

JCGM 100:2008, Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM).

ISO/IEC Guide 98, Expression of Uncertainty in Measurement.

EURACHEM/CITAC Guide “Quantifying Uncertainty in Analytical Measurement” Second edition (2000). A Williams, S L R Ellison, M Roesslein (eds.) ISBN 0 948926 15 5. Available from the Eurachem Secretariate.

- (C) Test results exceeding 0.3% THC.** Any sample test result showing with at least 95% confidence that the THC content of the sample is higher than the acceptable hemp THC level shall be conclusive evidence that one or more cannabis plants or plant products from the lot represented by the sample contain a THC concentration in excess of that allowed under the Act. If the results of a test conclude that the THC levels of a sample are conclusively higher than the acceptable hemp THC level, the laboratory will promptly notify the WSDA.
- (D) Retest Procedures.** Any hemp program licensee may request that the laboratory retest samples if it is believed the original THC concentration level test results were in error. If this occurs, the laboratory shall follow the same procedures as described in paragraphs (A)-(C) above that were followed to conduct the initial test. The licensee requesting the retest of the second sample will pay the cost of the test. The retest results shall be issued to the licensee requesting the retest and a copy shall be provided to WSDA.
- (E) Information Sharing with WSDA.** Laboratories performing THC testing for hemp produced under this program are required to share test results with WSDA. WSDA will provide instructions to all approved labs on how to electronically submit test results to WSDA.