Laboratory Guidance - Drying Field-Fresh Hemp Plant Samples in Preparation for Determination of Total THC on a Dry-Weight Basis

AOAC Cannabinoid Analytical Science Program (CASP) Working Group

1.0 Objective

The objective of this Appendix is to provide general guidance for laboratories to aid in the development of Standard Operating Procedures (SOPs) appropriate for processing hemp plant materials received fresh from the field. Field-fresh materials typically have a higher moisture content of 70-80% (1) requiring a preliminary drying step to reduce moisture content to a value that is sufficiently low (approx. 15%). Dried, milled samples are needed for requisite sample preparation by appropriate methods including AOAC 2018.11 for the determination of total tetrahydrocannabinol (THC) on a dry-weight basis.

2.0 Purpose and Scope

This document offers guidance to be used by laboratories to aid in the development of procedures to sufficiently dry field-fresh hemp material. The purpose of the procedure is to dry the material enough to facilitate adequate grinding and homogenization prior to analysis. The scope of this guidance is limited to the initial drying step only and is not intended to be used as the primary procedure used for sample preparation for determination and reporting total THC. Complete sample preparation details are described within published methods such as AOAC OMA 2018.11 (2).

In this context, the drying procedure needed and discussed herein is a preliminary step that, while representing an important step to ensuring that sample integrity is maintained, is not intended for use to directly generate final, reportable results for total THC (dry-weight basis).

It is critical for laboratories to develop a procedure in such a way that it can be applied in a consistent manner to minimize the introduction of measurement error as well as maintain sample integrity. During procedure development and validation, the laboratory should identify, evaluate and document critical steps and parameters of the final procedure, assess overall performance and address details that produce inconsistencies or errors in the final reported results for total THC (dry-wight basis). This document provides guidance with that documentation.

3.0 Introduction and Background – Drying Hemp Plant Materials

Requirements specified by the United States Department of Agriculture (USDA) Agricultural Marketing Service (AMS) for determination of total THC (dry-weight basis) in hemp explicitly state that hemp plant samples must be delivered to the testing laboratory as harvested from the field, i.e. in field-fresh condition (3). In this state and immediately post-harvest, the material will have a moisture content of between 70 – 80% and, while the USDA AMS rule does not mandate a specific drying process or procedure it does outline that testing laboratories "remove the majority of water" from hemp material prior to milling and homogenizing.

This initial drying step is crucial to the removal enough moisture from the plant material received by the laboratory to facilitate effective grinding and homogenization while not changing the material and possibly introducing analytical error. However, this step is not intended to take the material completely to dryness as it will undergo additional sample preparation prior to analysis for the determination of total-THC (dry weight basis). Rather, the target moisture content after this initial drying step is 10-15%. Moisture content significantly higher than this and the material will not mill properly. Moisture content less than 10% can result from overly aggressive drying and lead to change in cannabinoid profile and should also be avoided.

Consequently, the sample drying procedure is intended only to be used in preparing field-fresh hemp plant materials for subsequent regulatory analysis that reports total THC through full decarboxylation of tetrahydrocannabinolic acid (THC-A) or using the approved mathematical calculation for total THC shown below in Eq. (1):

$$\text{Total THC} = 0.877 * \text{THC-A} + \text{delta-9 THC} \qquad \text{Eq. (1)}$$

The drying procedure developed by the laboratory must be carefully crafted in order to minimize compromising the sample and introducing analytical error to the reported total-THC

value. There is no singular approach that will be acceptable for use by all laboratories due to a combination of diverse laboratory environmental conditions and available drying equipment. Each laboratory will be required to independently develop, evaluate and verify their own drying procedure.

There are three generally recognized mechanisms used for drying plant material: heat, air movement, and dehumidification. Any one or combination of these can and should be considered in the development of the laboratory drying procedure. It should be noted the each of these mechanisms must be well defined and controlled in the final procedure. For example, use of drying temperatures that are too high so as to shorten drying time should be avoided as these may cause decarboxylation of the acid forms of cannabinoids. The combination of drying temperatures that are too high and air movement can volatilize terpenes during the drying process further modifying the cannabinoid profile of the material. Both of these conditions will result in a change to the cannabinoid profile of the material and may introduce unwanted analytical error.

4.0 Hemp Drying Procedure - Experimental Design Guidance

This section provides guidance on collection of data needed to verify oven temperature and drying times are suitable for the drying of field-fresh hemp to a moisture content of less than 15% and appropriate for further laboratory processing prior to analysis. Drying conditions should be verified using procedures that are representative of routine sample processing, with consideration given to oven capacity and include various sample locations within the oven.

4.1 Study Protocol Development Considerations

The study Protocol should include all critical procedural details including sample handling, sample weighing, drying container types and handling and specific oven conditions. The Protocol should also include a comprehensive listing of all laboratory equipment needed to support the drying procedure such as types of weighing containers, required scales and balances as well as temperature monitoring equipment and timers. Scales, balances, timers and temperature monitoring equipment should all be calibrated prior to use. Data recording and processing software may also be identified. Customized algorithms developed to support data

collection and processing, e.g. Excel, should be evaluated and, if more complex, validated prior to use.

To assess the reproducibility of the final drying procedure, various container types (if used in that laboratory) and sample sizes representative of those typically received by the laboratory should be included to evaluate different drying times that may be required.

All details included in the study Protocol along with needed calculations should be captured in the final procedure document for laboratory personnel to use. Training, both initial and periodic, is also recommended.

4.2 Initial Sample Processing and Sub-sampling

A sample of field-fresh hemp may arrive at the laboratory in varied quantities and in different container types and size. Samples arriving to the laboratory in containers not suitable for drying should be transferred to containers that can withstand drying temperatures and handling conditions. Appropriate containers should be identified during procedure development and can be simple and may include, for example, paper bags. Larger sample quantities that cannot be dried in their original containers may be split and sub-sampled into more manageable sizes. Proper sub-sampling may be required and appropriate procedures must be established to ensure a representative sample is acquired using an established statistical sampling approach (4,5). During procedure development and validation, the impact of any sample handing or sub-sampling on sample integrity, including bias or other analytical error, should be formally assessed and included as a component of expanded measurement uncertainty for reporting total THC.

4.3 Sample Weighing and Drying Conditions

The recommended drying temperature is 65°C ± 5°C to facilitate reasonable drying times without compromising sample integrity. Using appropriate container type and sample quantity, the weight of the drying container along with the initial weight of the field-fresh sample are measured and recorded. The hemp sample is then placed in/on the weighing container and placed into the drying oven to begin the drying process.

During the study, the samples are periodically removed from the oven, weighed with the weight recorded and returned to the oven for further drying. This process is repeated until a constant weight is observed. Each weighing during the drying process can be made for the hemp material alone or a combined weight of the hemp and drying container. The same weighing scheme selected at the beginning of the drying procedure must be used throughout the drying process. Drying times should be carefully evaluated. The target moisture content using this procedure is 10-15% to ensure the material is adequately dry for grinding while still maintaining sample integrity for determination of Total THC.

As samples are withdrawn from the oven and weighed, loss on weighing is calculated relative to the previous weighing to monitor drying progress. Table 1 provides guidance on how often to weigh the sample when developing appropriate drying methods.

Table 1. *Suggested periodicity for collecting weights to determine moisture-loss curve for field-fresh hemp.*

Hemp Sample Weight, g	Weighing Frequency
< 100	6x / Day
100 - 500	3x / Day
> 500	1x / Day

The study should continue with sample weight until at least two successive weight determinations produce a percent weight change of less than 5%. If samples and containers are weighed together during the study, percent weight-change, %-Wt Change, is calculated using Eq. (2) below:

$$\%\text{-Wt Change} = (TW_p - TW_c) / (TW_p - W_{cont}) \times 100 \qquad \text{Eq. (2)}$$

Where:

TW_p = Total Weight of hemp sample + container at previous time point, in grams

TW_c = Total Weight of hemp sample + container at current time point, in grams

W_{cont} = Weight of drying container, in grams

Note that Eq. (2) requires the weighing of the container used for drying the hemp sample.

If only sample weights are measured during drying, percent weight-change is calculated using a simplified version of Eq. (2) shown below in Eq. (3):

$$\% \text{ -Wt Change} = (SW_p - SW_c) / (SW_p) \times 100 \qquad \text{Eq. (3)}$$

Where:

SW_p = Sample Weight of hemp at previous time point, in grams

SW_c = Sample Weight of hemp at current time point, in grams

In addition, manual evaluation of the dried sample should also suggest that the material will readily crumble when handled. If not, the sample is not dry enough for grinding and should be dried for a longer period of time. Larger sample quantities or those with higher moisture content will require more time to dry than smaller, drier sample quantities.

Similarly, the total percent moisture removed for a given drying interval relative to the final weighed sample that has reached steady-state can be estimated by using Eq. (4) for sample and drying container weighed together or using Eq. (5) if weighing the sample alone and without including the drying container. As above in Eq (2), Eq (4) requires the weighing of the container used for drying the hemp sample:

$$\%\text{-Moisture Loss} = (TW_c - TW_f) / (TW_c - W_{cont}) \times 100 \qquad \text{Eq (4)}$$

Where:

TW_c = Total Weight of hemp sample + container at current time point, in grams

TW_f = Total Weight of hemp sample + container at final time point, in grams

W_{cont} = Weight of drying container, in grams

$$\%\text{-Moisture Loss} = (SW_c - SW_f) / (SW_c) \times 100 \qquad \text{Eq (5)}$$

Where:

SW_c = Total Weight of hemp sample at current time point, in grams

SW_f = Total Weight of hemp sample at final time point, in grams

4.4 Example Studies

Data from two example studies are presented in Table 2. The data obtained were collected for 140 g and 400 g samples of field-fresh hemp oven-dried at 60°C using two different container types, closed paper bags and a drying pan.

Table 2. *Example Data for 140-g and 400-g field-fresh hemp samples oven-dried at 60ºC in closed paper bags or open pan.*

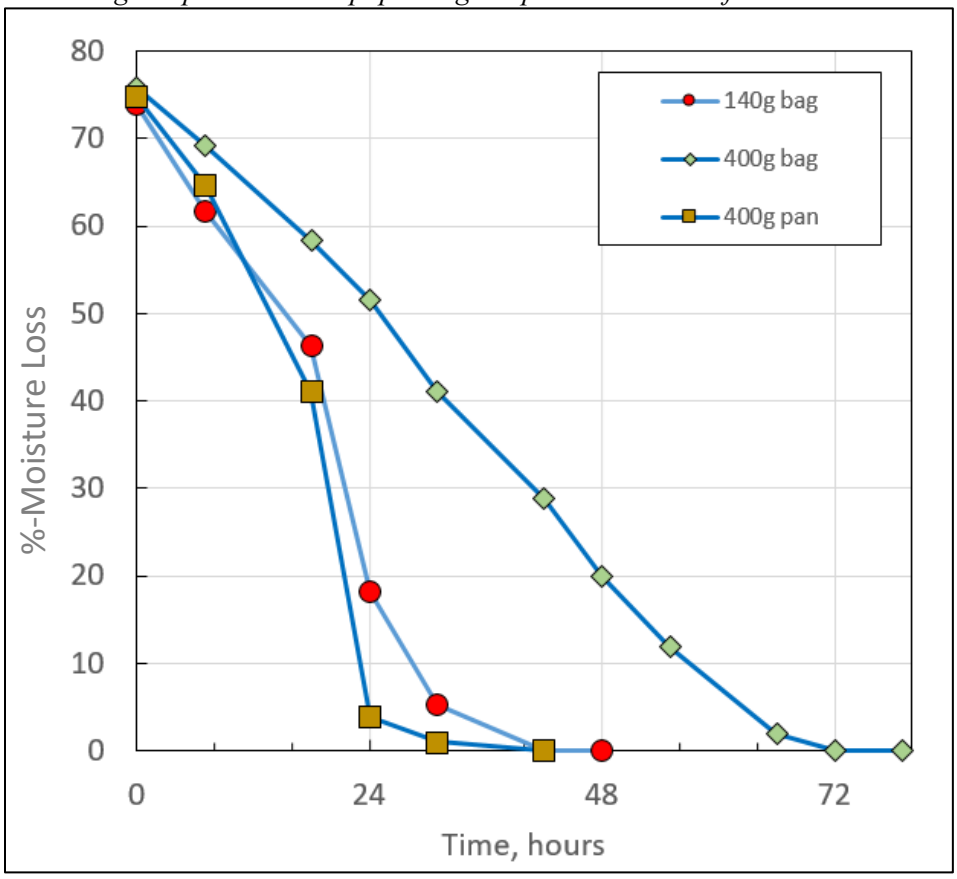
Time, h	Measured Weight, g	Weight Change[1], %	%-Total Moisture Loss[2]
---------- 140-g Hemp Sample Dried in Paper Bag[3] ----------			
0	161	-	74
7	117	27	62
18	90	23	46
24	67	26	18
31	61	9	5
42	59	3	0
48	59	0	0
--------- 400-g Hemp Sample Dried in Paper Bag[3] ----------			
0	420	-	76
7	334	22	70
18	254	26	59
24	222	14	52
31	186	18	42
42	158	17	30
48	143	11	21
55	132	9	13
66	121	10	3
72	119	2	1
79	118	1	0
---------- 400-g Hemp Sample Dried Using Drying Pan[3] ----------			
0	932		75
7	820	29	65
18	708	40	41
24	643	39	4
31	640	3	1
42	639	1	0

[1] Percent weight change per Eq. (2) or Eq. (3).
[2] Percent moisture per Eq. (4) or Eq. (5).
[3] Container weights – Paper Bag: 23 g; Drying Pan: 540 g.

For the examples shown above using a drying temperature of 60°C, 79 hours was needed to adequately dry a 400-g hemp sample using a closed paper bag, i.e., to reach constant weight defined as <5% weight change on two successive weighings indicating that no more moisture was being removed from the sample. Conversely, only 24 hours drying time was required for a 400-g sample dried using an open pan. Finally, 48 hours drying time was required for a 140-g sample dried in a closed paper bag.

The rate of moisture loss can be visualized by calculating %-Moisture Loss vs the last sample weighing and plotted vs weighing interval times as shown in Figure 1. Moisture losses of 0% are calculated for samples in which no more moisture is removed in drying and suggest drying is complete. Samples can be expected to have variable moisture content when removed from the oven and the material may in fact absorb some small amount of moisture prior to or during milling at laboratory ambient conditions.

Figure 1. *Example Moisture-loss curves for field fresh hemp at 60°C for 140-g and 400-g samples in closed paper bags or pan. See Table 1 for data used.*



5.0 Summary and Conclusions

The goal of drying field-fresh hemp samples is not to remove all the moisture from the samples but rather to reduce the moisture content of the plant material to a level appropriate for grinding the material into a powdered analytical sample in preparation for analysis. The target moisture content of the dried material is approximately 10-15% and is readily apparent when the dried plant material disintegrates readily when crushed. Moisture content significantly higher than this and the material will not mill properly. Moisture content less than 10% can lead to change in cannabinoid profile and should also be avoided.

This guidance is intended to assist laboratories in developing and validating their internal SOP for processing field fresh hemp to a dried state appropriate for milling and subsequent preparation of an analytical sample for total THC analysis. To report percent total THC on a dry-weight basis, an additional and separate analytical determination of moisture must be made on a test portion of the sample taken at the same time a test portion is analyzed for total THC in accordance with validated methods (see OMA 2018.11).

6.0 References

(1) "Cannabis Indoor Growing Conditions, Management Practices, and post-Harvest Treatment: A Review"; D. Jin' S. Jin, J. Chen, American Journal of Plant Sciences; Vol 10 (6), 2019

(2) OMA 2018.11

(3) Laboratory Testing Guidelines, US Domestic Hemp Production Program, Jan 15, 2021

(4) AOAC INTERNATIONAL, OMA Appendix K: Guidelines for Dietary Supplements and Botanicals; Section 2.1, "Preparation of the Laboratory Sample".

(5) "Regulatory sampling of industrial hemp plant samples (Cannabis sativa L.) using UPLC-MS/MS method for detection and quantification of twelve cannabinoids"; C.R. McCurdy, et al, Journal of Cannabis Research. 2, Article 42 (2020)