Title: **Determination of Total Moisture by the CDC Method**

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Release / Revision Record for SOP

Status (Initial/Revision/Retired)	Document Revision Number	Issue/Revision Date	Revision Identification	Revision Author
Initial Release	1	08/08/2014	Original Issue – PPI converted to SOP format.	Tammy Blake
Revision	2	02/06/2017	Revision includes portioned product, whole cigar, and pipe tobacco sample preparation, sample handling requirements, and testing procedure for FDA reporting.	Marc Krauss

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A. SCOPE

1. This procedure documents the process for determining Total Moisture in tobacco products as measured by the loss of compounds volatilized under testing conditions.
2. This procedure is applicable to tobacco samples ground to less than 4 mm particle size. Results will be reported in weight percent total moisture.
3. This method is used to determine total moisture of smokeless tobacco products for regulatory CDC reporting. This method also provides instructions to determine total moisture of smokeless tobacco products, whole cigars, and pipe tobacco for FDA reporting.

B. DEFINITIONS

1. Total Moisture – The amount of volatiles lost after 3 hours in a forced-air oven set at 99 ± 1°C. For this procedure the terms Total Moisture and Moisture are equivalent to the term Oven Volatile (OV) used within the laboratory documentation and references: i.e., “Total Moisture by CDC Method” is equivalent to “OV by CDC Method”.
2. Weighing Moisture Dish – aluminum dish with lid used for weighing and drying samples that is placed into the oven for total moisture determination.
3. Internal Quality Control Sample (IQCS) – homogenized tobacco sample (monitor sample) with an established target and control limits used to monitor performance of the analysis.
4. CDC OV of pouched smokeless tobacco products for CDC reporting – the pouches are opened, the papers are discarded and OV is determined on the tobacco only.
5. CDC OV of pouched smokeless tobacco products for FDA reporting – the pouches are opened and both the tobacco and paper are added to moisture dishes and then mixed for OV determination.
6. CDC OV of whole cigars for FDA reporting – OV may be requested to be determined on non-equilibrated or on equilibrated product. After removal of tips (if present), the cigars are cut into thirds and the cigar pieces are added to the moisture dish for OV determination.
7. CDC OV of pipe tobacco for FDA reporting - OV may be requested to be determined on non-equilibrated or on equilibrated product. The pipe tobacco is added to moisture dishes for OV determination.

C. RESPONSIBILITIES

1. The designated trained analyst performing the method is responsible for following all steps of the procedure and documenting and reporting any procedural deviations from the method to laboratory management.

2. Personnel using this test method are responsible for conducting the analysis in a manner consistent with the safety policies of ALCS.

D. VALIDATION

1. This procedure is based on "A Uniform Protocol for the Analysis of Nicotine, Total Moisture, and pH in Smokeless Tobacco Products" from CDC published in the 2009 Federal Register.
2. Validation is not required for analysis of products within the intended scope of the CDC protocol (e.g., snus, moist snuff, etc.) However, the laboratory must demonstrate performance by documenting:

   a. tests performed by trained personnel
   b. a monitored quality control program
   c. inter-lab studies with demonstrated proficiency and
   d. periodic audits of the procedure

3. Tobacco products or product configurations not explicitly defined in the CDC protocol but capable of being prepared as described in the document can be analyzed using the CDC protocol (i.e., ground to a particle size of < 4 mm.) The laboratory must demonstrate performance as described previously.
4. (b) (4)

Average	Std Deviation	%RSD
(b) (4)		

5. Cigars are not mentioned in the CDC protocol; however, the preparation and validation of this matrix are documented in ST-TM-440-204 Determination of Volatiles by Mechanical Convection Oven and in ST-TM-440-204_07 Supplemental Validation Report (Cigars).
6. Pipe tobacco is not mentioned in the CDC protocol, and is not documented in this validation; however, pipe tobacco is considered to be in scope because the cigar fillers used in the validation contain pipe tobacco. The preparation and validation of the cigar matrix is documented in ST-TM-440-204 Determination of Volatiles by Mechanical Convection Oven and in ST-TM-440_07 Supplemental Validation Reports (Cigars).

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E. EQUIPMENT AND APPARATUS

1. Equipment Requirements and Apparatus

   a. Analytical balance, 0.0001g Readability; e.g. Mettler Toledo XS204, or equivalent.
   b. Desiccator – implosion proof container large enough to hold 20 moisture dishes with lids.
   c. Oven Gloves – insulated for working with hot surfaces.
   d. Oven – Fisher Isotemp® forced-air, cat# 15-103-0511, or equivalent.
   e. Brush – Wooster Golden Glo, blended nylon and polyester 2" paint brush, or equivalent.

   f. Weighing Moisture Dishes, aluminum moisture dishes with close fitting slip covers - approximately 2½" diameter x 1¼" - 2½" height, available from Dual Manufacturing, Chicago, IL; part#AD-6344.

      Note: Dishes and the corresponding lids are numbered manually with non-toxic permanent ink. Newly marked dishes should be baked in an oven at 100°C for three hours prior to use. Dishes and lids should be washed annually or as required to remove tobacco residue, and then renumbered and baked. Record wash and bake information on the Dish Maintenance Form.

2. Instrument Setup

Oven Temperature	99 ± 1.0°C

Note: If the oven is not at the target temperature, allow the oven to come to temperature before placing any samples in the oven. If the oven fails to come to temperature, notify lab management. Ovens must be free of debris as this can affect air flow which will affect the measurement of total moisture.

F. CHEMICALS AND REAGENTS

1. Desiccant – Drierite™, anhydrous calcium sulfate, non-indicating (white) and indicating (blue) from WA Hammond, Drierite Company, LTD, parts #13005 and #23005.

G. SAMPLE REQUIREMENTS

1. Sample Requirements

   a. Smokeless tobacco products are delivered to the laboratory in Nalgene bottles prepared per WI 097-1108 Sample Preparation.
   b. For samples stored in the freezer or refrigerator, sufficient time should be given to allow the sample material to reach room temperature before sample preparation. A minimum of 24 hours is required for the samples to equilibrate

after being removed from the freezer. The products must not be opened during equilibration

c. Mix IQCS sample prior to use. Duplicate monitor dishes are prepared per oven load following the sample procedure.

H. PROCEDURE

1. Calibration

   a. Check the balance calibration in accordance with SOP 095-9483 Calibration Verification.
   b. The oven calibration for temperature is coordinated by lab personnel and scheduled with Lab Services in accordance with the IM&TE schedule.
   c. Desiccant:

      1) It is a combination of non-indicating (white desiccant) and indicating (blue desiccant). The blue desiccant must be visible and inspected for saturation prior to using the desiccator. If the majority of the indicating desiccant has turned from blue to any shade of purple, replace the desiccant. (Desiccant in shades of pink indicates an extreme state of saturation.) Record the date of desiccant replacement on each desiccator.
      2) Use the following amounts when replacing the desiccant: combine approximately one quart of non-indicating (white) desiccant and approximately three tablespoons of indicating (blue) desiccant in each desiccator.

2. Sample Handling

   This section provides specific instructions on sample handling for the different tests and product categories; however, Section H.3 provides instructions for creating a LIMS batch and specifically when the tobacco product is added to the moisture dish (i.e. after the empty container weight is measured).

   a. CDC OV Reporting for Smokeless Tobacco Products

      1) All tobacco samples must be less than 4 mm particle size.
      2) (b) (4) must be prepared as described in WI 097-1108.
      3) For pouched products, the pouches are opened and the papers are discarded. OV is determined on the tobacco only.
      4) Two replicates are determined for each CDC smokeless tobacco sample.

   b. FDA OV Reporting for Smokeless Tobacco Products

      1) All tobacco samples must be less than 4 mm particle size.
      2) (b) (4) must be prepared as described in WI 097-1108.

3) For pouched products, OV is determined on the tobacco and the paper. In the OV dish, weigh enough intact portioned products (tobacco with pouch) to achieve a weight between 4.5 - 5.5g. Be sure to use the whole portion products. Next, separate the tobacco from the pouch, re-combine, then analyze together.

4) Three replicates are determined for each FDA smokeless tobacco sample.

c. FDA OV reporting of Whole Cigars

1) Cigar OV may be requested to be determined on **non-equilibrated** cigars or on **equilibrated** cigars. Whether or not the cigars are equilibrated before OV determination must be stated in the test request. Cigars that require equilibration prior to OV determination shall be equilibrated as described in SOP 095-0029 “Sample Handling and Conditioning”.
2) Cigar OV is determined on the entire rod, with the plastic, wood, or cellulose acetate (filter) tip removed. The OV measurement includes the filler and the tobacco wrapper or wrapper\binder.
3) The cigars should be removed from all packaging immediately prior to testing. If present, cigar bands should also be removed prior to testing.
4) Remove plastic or wood tips by cutting the tip off using a razor blade. Remove filter tips by cutting the tip off flush with the tobacco column using a razor blade.
5) Each replicate requires a sufficient number of cigars to reach the pre-analysis target weight of 4g – 8g. The following information may be used as a guide; however, the number of cigars used for each determination may need to be adjusted:

a) [redacted]: 1 cigar with approximately 1 inch of the tapered end cut off in order to reduce the weight to < 8g.
b) Standard length wood tip, plastic tip, and filter tip cigars: 2 cigars
c) [redacted] Cigars (110mm): 2 cigars
d) [redacted] Cigars (85mm): 3 cigars
e) [redacted]: 4 cigars

6) Cut or break the cigars into thirds and deposit the cigars into the OV dish. The cigars may be cut or broken into smaller pieces if necessary to fit in the into the OV dish.

7) Three replicates are determined for each FDA cigar sample.

d. FDA Reporting of Pipe Tobacco

1) Pipe Tobacco OV may be requested to be determined on **non-equilibrated** tobacco or on **equilibrated** tobacco. Whether or not the cigars are equilibrated before OV determination must be stated in the test request. Pipe tobaccos that require equilibration prior to OV determination shall be equilibrated as described in SOP 095-0029 “Sample Handling and Conditioning”.
2) Three replicates are determined for each FDA pipe tobacco sample.

3. Create an Oven Batch in LIMS

a. Prepare monitor dishes and enter weights in LIMS.
b. Prepare the test sample(s) and enter weights in LIMS.

1) Enter the first dish number, always starting with the lowest odd-numbered dish.
2) Ensure moisture dish and balance weighing surfaces are clean.
3) Zero the balance and place an empty moisture dish with lid on the balance tray to obtain the container weight. Tare the balance.
4) Gently mix the tobacco by inverting the container several times, prior to filling the dish. Weigh 5.0 ± 0.5g of sample into each dish. Cover with lid when removing from the balance.

Note: If LIMS is out of operation, the steps listed above may be performed manually using the Form 099-3107 OV Manual Calculation Sheet.

4. Analysis

Caution: Always wear protective gloves (insulating type) when transferring dishes in and out of the oven. Portable sleeves are available for operators who desire to use them.

a. Load the samples in the oven:

Note: Moist and Dry products (e.g. moist snuff and dry snuff) are NOT to be tested in an oven at the same time. Cigars/ pipe tobacco and smokeless tobacco are not to be tested in an oven at the same time nor should these sample types be tested with any other types of tobacco or tobacco products.

1) Every oven load must consist of exactly 20 dishes: 2 monitor dishes and 18 test sample dishes. If less than 9 samples are available for testing, empty dishes with the lids must be used to ensure that a total of 20 cans are in the oven for each batch.

2) Prior to opening the oven door, record the oven temperature on the CDC Oven Log Worksheet. If the oven is not within temperature limits, contact management.
3) Remove the lid from a sample or monitor dish, place it upside down under the dish, and place the dish on the oven shelf tray.
4) Using a 4 by 5 array, place sample dishes randomly in the oven. The two monitor dishes are placed diagonally opposed from each other in the middle. An example placement is shown in Figure 1 below.

Sample	Sample	Sample	Sample	Sample
Sample	IQCS	Sample	Sample	Sample
Sample	Sample	Sample	IQCS	Sample
Sample	Sample	Sample	Sample	Sample

Figure 1. Example Dish Layout

5) Once the oven is loaded, close the door. Record the time the oven was loaded to the nearest minute on the CDC Oven Log worksheet (099-3114). Attach a label that reads "Oven In-Use, Do Not Open Door."

b. Unloading the oven:

1) Before unloading the oven, ensure the red stopper on the side of the desiccator lid is in place and secure. Check that the o-ring is in good condition and has an applied layer of silicone grease.
2) Remove samples from the oven after 3 hours ±5 minutes.
3) Just prior to opening the door to remove samples, record the oven temperature to the nearest minute on the CDC Oven Log worksheet.
4) Replace the lid on each dish as it is removed from the oven and place each dish in the desiccator. Close the desiccator after all dishes have been loaded. Do not include empty dishes.
5) Allow the moisture dishes to cool to room temperature. This takes approximately thirty to forty-five minutes. After the cooling period, touch the surface of the dishes to ensure adequate cooling has occurred prior to weigh-back. Samples are to be removed and weighed as described below as soon as practical after they have cooled. Dishes must re-weighed before the end of the shift.

c. Reweigh Samples:

Note: All dishes in the desiccator may be removed at the same time providing all weighing is completed within five minutes from the time the dishes are removed. Keep desiccator covered at all times except to remove dishes for weighing.

1) Record the time the samples are removed from the desiccator on the CDC Oven Log Worksheet.
2) Reweigh samples and enter results into LIMS.
3) Evaluate the mean for the two IQCS samples for quality control per Section H.4. Save the batch.
4) Discard the contents of the moisture dishes and clean dishes with a brush.
5) Prepare a regulatory packet for review and approval by authorized personnel.
6) After the regulatory packet has been authorized, store data in accordance with SOP 095-0037 Results Verification.

5. Quality Control

a. Prior to loading and removing dishes, check the oven temperature and initiate corrective action if temperature is outside acceptable range.

b. An Oven Check (verification) is required

1) following maintenance before the oven can be used for sample testing, or

2) when the method is out of out of statistical control, as determined by IQCS control chart evaluation, without a known root cause.

3) Following the test procedure, weigh 10 dishes of a freshly opened IQCS sample. Place the dishes in the oven with 10 empty dishes. If the results are acceptable per lab management, release the oven into service.

c. The IQCS is analyzed in duplicate in each oven batch. Plot the average of the two monitors on the QI Macros Control Chart and evaluate against the limits determined for the monitor. The rules and information on how to address out-of-control conditions are documented on the control charts.

1) If the IQCS indicates that the method is out of statistical control, reanalyze the IQCS once to identify if the root cause is the IQCS measurement.

2) If the oven produces an average IQCS result that is still out of statistical control, place the oven out-of service and contact authorized maintenance personnel. The oven must pass the Oven Check prior to release back into service.

6. Calculations

   Calculations are performed in LIMS. The percent total moisture for each dish is calculated as:

$$\text{Total Moisture (\%)} = \frac{\text{Sample Wt.}_{\text{Original}} - \text{Sample Wt.}_{\text{After Drying}}}{\text{Sample Wt.}_{\text{Original}}} \times 100$$

I. REFERENCES

1. Validation of Oven Volatiles (OV), Precision Model Ovens. August 27, 2010. In support of test method ST-TM-440-204.
2. ST-TM-440-204 Determination of Volatiles by Mechanical Convection Oven.
3. ST-TM-440-204_07 Supplemental Validation Report (Cigars).
4. Department of Health and Human Services, Centers for Disease and Prevention, Revised Protocol for Analysis of Nicotine, Total Moisture, and pH in Smokeless Tobacco Products, Federal Register / Vol. 74, No. 4 / Wednesday, January 7, 2009 / Notices, pages 712 – 719.
5. SOP 095-0061Generalized Procedure for Determining Uncertainty.
6. SOP 095-0029 Sample Handling and Conditioning
7. WI 097-1108 Sample Preparation
8. SOP 095-0037 Results Verification

J. FORMS

1. 099-3106 Dish Maintenance Form
2. 099-3107 OV Manual Calculation Sheet
3. 099-3114 CDC Oven Log Worksheet