Title: **Measurement of Water Activity in Tobacco and Tobacco Products**

Quality System Compliance Review and Approval

Approved By:

Tammy L. Blake	**Quality Coordinator, Analytical Sciences**

Signature: Tammy L. Blake
Digitally signed by Tammy L. Blake
DN: cn=Tammy L. Blake, o=Analytical Sciences ID 47286, ou=ALCS/Analytical Sciences, email=Tammy.L.Blake@altria.com, c=US
Date: 2017.01.24 08:19 53 -05'00'

Management Review and Approval

Approved By:

Georgios D. Karles	**Director, Analytical Sciences**

Signature: Georgios D. Karles
Digitally signed by Georgios D. Karles
DN: cn=Georgios D. Karles, o=Analytical Sciences ID 00071009, ou=Analytical Sciences, email=georgios.d.karles@altria.com, c=US
Date: 2017.01.24 11:20:28 -05'00'

Subject Matter Expert Review Team

Robert Ragland

Robert Ragland
Digitally signed by Robert Ragland
DN: cn=Robert Ragland, o=Aerosol & Material Science, ou=Regulatory Sciences, email=Robert.B.Ragland@Altria.com, c=US
Date: 2017.01.23 14:59:15 -05'00'

Mark Rusyniak

Mark Rusyniak
Digitally signed by Mark Rusyniak
DN: cn=Mark Rusyniak, o=Altria Client Services, ou=Analytical Sciences, email=mark.j.rusyniak@altria.com, c=US
Date: 2017.01.24 08:06:17 -05'00'

Title: **Measurement of Water Activity in Tobacco and Tobacco Products**

Release / Revision Record for SOP

Status (Initial/Revision/ Retired)	Document Revision Number	Issue/Revision Date	Revision Identification	Revision Author
Initial	1	10/20/2014	Transfer from PPI to SOP template	Amy McFarlane
Revision	2	1/31/2017	Rename and revise to include cigar tobacco and (b) (4) products and to include the Decagon's Aqualab TDL and removes the TEV instrument.	Robert Ragland

Title: **Measurement of Water Activity in Tobacco and Tobacco Products**

Table of Contents

Title: **Measurement of Water Activity in Tobacco and Tobacco Products**

A. SCOPE

1. This method describes the procedure for using Decagon's Aqualab water activity meters equipped with tunable diode laser detection (TDL) to measure water activity of tobacco products.
2. This test method applies to tobacco products having water activities between 0.000 and 1.000 water activity units. Data will be collected for samples having water activities below the lowest verification standard ($0.25A_W$); however, these data will be reported as less than the LOQ.
3. This method has been shown to be valid for loose and portioned smokeless tobacco, tobacco filler (cigarette/cigar), and (b) (4)

B. DEFINITIONS

1. **Water Activity** - Water activity or A_w is the ratio of the partial pressure of water in a substance to the partial pressure of pure water at the same temperature.
2. **Sensor Drift** – Sensor drift is a systematic bias in the instrument output caused by interfering compounds in a sample.

C. RESPONSIBILITIES

1. The analyst must be familiar with the operation of a Decagon Devices Inc. AquaLab TDL water activity meter.
2. The designated trained analyst performing the method is responsible to follow all steps of the procedure and to document and report any procedural deviations from the method to laboratory management.
3. Personnel using this test method are responsible for conducting the analysis in a manner consistent with the safety policies of ALCS.

D. VALIDATION

Validation work was previously performed for various tobacco products including MST, SNUS, and Cigar. Previous validations utilized a Decagon Devices AquaLab 3TE or 4TEV water activity meter to perform the work at the time however the matrices were also tested on the TDL unit as well to ensure previous validation references are in agreement with current instrumentation. Current validation work was performed to incorporate Verve products into the smokeless category of tobacco products as well as ensure there were no matix interferences with the new instrument.

Title: **Measurement of Water Activity in Tobacco and Tobacco Products**

E. EQUIPMENT AND APPARATUS

1. Equipment and Apparatus Required
   a. Water Activity Meter, AquaLab Series TDL available from Decagon Devices, Inc., Pullman Washington.
   b. Sample cups with lids, disposable; Decagon Devices, Inc. p/n: 40107 and 40116.
   c. AquaLab Cleaning Kit consisting of cleaning solution, plastic cleaning spatula, Kimwipes®, activated charcoal pellets; available from Decagon Devices, Inc., p/n: 30595.
   d. Ziplock Mylar bag (p/n 5126297) or similar storage container.
2. Instrument Setup
   a. The AquaLab Water Activity meter should be in an area away from air conditioner and heater vents, refrigerator or oven exhausts, or other items that may cause sudden rapid temperature fluctuations. See Chapters 3 and 9 of the Aqualab TDL Operator's Manual for further discussion.
   b. The meters are temperature controlled. A complete and accurate reading will not be made until the sample is within 2 ºC of the instrument set point. Instrument cycle time may be reduced by maintaining the laboratory temperature near the meter's set point temperature (25.0 ºC).
   c. Set the meter's set point temperature to 25.0 °C and the temperature equilibration range to ± 0.5 ºC.
   d. Turn on the AquaLab Water Activity Meter at least 30 minutes prior to measuring water activity. The meter may be left on unless no future testing is planned.
3. Instrument Maintenance
   a. If visual inspection of the sample chamber indicates contamination or is otherwise dirty, perform sensor cleaning using the cleaning kit provided by Decagon as outlined in the instrument manual.
   b. If water activity standards read outside of the specified ranges, the analyst should run one run cycle of the activated charcoal pellets. After the activated charcoal pellet has been run, the verification solutions must be reanalyzed for acceptance. The pellets may be reused unless they get wet. If this procedure does not resolve the failing verification solutions, refer to chapter 7, Verification and Calibration, of the owner's manual for maintenance procedures. If the problem is not resolved following the outlined procedure, contact laboratory management for further instructions before continuing.

c. The operator must consult lab management, the ALCS SME, or the instrument manufacturer if the water activity standards continue to read outside of the specified range to determine if a multi-point calibration needs to be conducted.

d. Annual sensor calibrations must be performed by Decagon Devices, Inc.

F. CHEMICALS AND REAGENTS

1. Chemicals Required

   a. Performance Verification Standards with the following water activity and salt concentrations (available from Decagon Devices, Inc., Pullman, Washington):

      1) 1.000 (USP purified water); Decagon Devices, Inc. p/n: 40464

      2) 0.920 (2.33 M NaCl); Decagon Devices, Inc. p/n: 40465

      3) 0.760 (6.0 M NaCl); Decagon Devices, Inc. p/n: 40460

      4) 0.500 (8.57 M LiCl); Decagon Devices, Inc. p/n: 40461

      5) 0.250 (13.41M LiCl); Decagon Devices, Inc. p/n: 40462

   b. Activated Charcoal, one cup comes with each Cleaning Kit, Decagon Devices Inc. p/n: 30595

   c. Drierite®, anhydrous calcium sulfate desiccant, or similar desiccant.

G. SAMPLE REQUIREMENTS

1. Prior to opening the sample container, allow samples to equilibrate to the temperature of the lab in which they will be tested prior to starting.

H. PROCEDURE

1. Sample Handling

   Note: the validation results showed that water activity results could change by more than 10% if the samples were left open to ambient conditions. For this reason, samples must be immediately sealed in the original container after removing an aliquot for analysis. If the sample container will be opened more than three times to remove samples for analysis, the sample should be aliquoted into smaller containers (e.g. ziplock Mylar bag) that are impervious to water migration prior to testing.

   a. MST/Snus/Cigar Filler/ (b) (4)

      1) Add samples to the sample cups by pouring them in, if possible, or using spatulas or forceps. If it is necessary to handle the sample for any reason, gloves should be worn. Once inside the sample cup, the sample may be manipulated with spatulas or forceps.

2) The sample should cover as much of the bottom of the cup as possible.

3) Do not add sample above the line on the sample cup. An overfilled sample cup may contaminate the chamber sensors.

4) Measure samples immediately after opening the storage container and filling the cup. If samples are left out in ambient lab conditions for more than 5 minutes they may adsorb or desorb moisture and give a false value. Samples must be stored in a manner adequate to protect from atmospheric changes at all times, e.g. sealed containers with lids, zip locked Mylar bags, original packaging, etc. It is best practice to aliquot from the storage container and reseal the container immediately to preserve the product in its packaged state.

5) In cases where more than three sample aliquots are to be tested from a single container, the repeated opening and closing of the container may affect the water activity of the samples. In cases such as this, samples should be aliquoted into suitable separate containers (e.g. ziplock Mylar bag) to minimize repeated exposure to the atmosphere.

(b) (4)

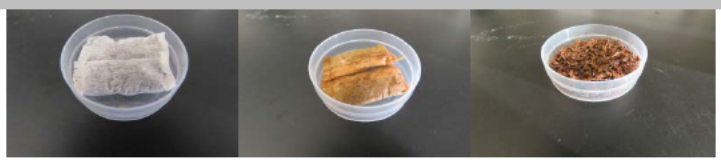

Figure 1. Orientation and placement of tobacco products in sampling cups. From left to right: (b) (4) SNUS, Pouched MST, Loose MST/Cigar Filler.

2. Calibration Verification

a. Select Decagon A_w verification standards with levels bracketing the expected water activity levels of the samples. If the expected A_w levels are unknown, analyze all four verification standard levels: 0.250, 0.500, 0.760, and 0.920. Analyze the verification standards from lowest to highest to minimize sensor hysteresis. (b) (4)

(b) (4)

1) Open the ampoule of the desired verification standard and transfer it into a sample cup.
2) Move the chamber lever to the open position and lift the chamber lid.
3) Carefully place the cup in the chamber, close the lid and move the lever to the "Read" position.
4) Once the reading is complete, the meter will beep and display the water activity value, reading time and temperature. Record the verification standard result on the water activity sample worksheet.
5) The verification standards must read within ± 0.005 units of their nominal A_w value.

b. Continue reading the remaining verification standards and evaluate whether the meter and sensor are suitable for use.
c. If the calibration verification does not pass, refer to Section E.3 of this document.

3. Analysis
   a. Verify the calibration of the instrument using the vendor certified verification standards as discussed in section H.2. Calibration. A minimum of two verification solutions must be used and the verification standards must bracket the sample A_w. If the sample A_w falls below 0.250 verification solution, the two lowest verification solutions (0.250 and 0.500) should be analyzed.
   b. Unless otherwise specified, each sample is analyzed in triplicate.
   c. Add sample to the sample cup
      1) For MST/Snus/Cigar Filler samples – Add sample until the cup is one-third to one-half full. A ring on the inside of the cup marks one-half full. Two pouches should be used for analysis of SNUS or MST pouches.
      2) For (b) (4) samples – Add samples until the bottom of the cup is covered. For (b) (4) this would consist of 5 pieces per cup (b) (4) – 3 pieces per cup, (b) (4) – 4 pieces per cup, (b) (4) (b) (4) – 4 pieces per cup. Note, with proper orientation, the (b) (4) samples may be above the one-half ill line (see Figure 1).
   d. Move the chamber lever to the open position and lift the chamber lid.
   e. Carefully place the cup in the chamber, close the lid and move the lever to the "Read" position.

f. Once the reading is complete, the meter will beep and display the water activity value, reading time and temperature.

g. Record the value for the sample on the water activity worksheet.

4. Run verification standards prior to and following the measurement of samples. Measure two verification standards that bracket the sample water activity levels every 6 to 9 sample measurements (2 or 3 samples analyzed in triplicate) or less. All samples must be bracketed by passing verification standards. Refer to section E.3.b. if the verification solutions fail the acceptance criteria.

5. Calculations and Reporting

   a. Record water activity results, reading time, and temperature readings of verification standards and all samples on the water activity worksheet.

   b. Enter data manually into LIMS.

6. Quality Control and Acceptance Criteria

   a. The validity of the instrument calibration must be checked routinely during an analysis batch by analyzing verification standards that bracket the samples every 6 to 9 sample replicates. However, it is recommended that only 6 replicates be analyzed between verification solutions for samples that are highly volatile (flavored MST). If verification standards fail specification, all samples analyzed since the last passing verification standards are suspect and must be reanalyzed.

   b. If the sample A_w falls below 0.250 verification solution, the two lowest verification solutions (0.250 and 0.500) should be analyzed.

   c. The verification standards must read ± 0.005 units of their nominal A_w value on the TDL instrument.

I. REFERENCES

1. *Measurement of Water Activity in Smokeless Tobacco Products*, Test Method Validation Report, March 22, 2007
2. *Supplemental Validation SOP 096-3021 "Measurement of Water Activity in Smokeless Tobacco"*, Test Method Validation report, October 10, 2014
3. *Measurement of Water Activity in Cigar Filler Supplemental Validation*, Test Method Supplemental Validation Report, June 5, 2015
4. *Measurement of Water Activity in Tobacco and Tobacco Products*, Test Method Validation Report, January 17, 2017
5. Qualification of Decagon Devices TDL Water Activity Meter, Regulatory Sciences Report, January 17, 2017

6. Verification of sample size for pouched smokeless products, Regulatory Sciences Report, January 17, 2017
7. AquaLab Water Activity Meter, Operator's Manual: For AquaLab Model TDL, Decagon Devices Incorporated

J. FORMS

1. Water Activity Results Worksheet