Title: **Determination of pH by the CDC Method**

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=Analytical Sciences, email=Tammy.L.Blake@altria.com, c=US
Date: 2018.02.26 11:17:51 -05'00'

Management Review and Approval

Approved By:

Yezdi B. Pithawalla	**Director, Analytical Sciences**

Signature: Yezdi B. Pithawalla

Digitally signed by Yezdi B. Pithawalla
DN: cn=Yezdi B. Pithawalla, o=Analytical Sciences ID 00050545, ou=ALCS/ Analytical Sciences, email=yezdi.b.pithawalla@altria.com, c=US
Date: 2018.02.26 11:59:03 -05'00'

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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.	Jim Fisher
Revision	2	07/14/2017	This SOP is a re-write. Revision includes addition of tobacco products, product sample preparation, and testing procedure. References added. Administrative changes for clarity.	Marc Krauss/ Hui Liu/ Anthony Brown
Revision	3	02/27/2018	Administrative change due to a misprint.	Natasha Shelton

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A. SCOPE

1. This procedure documents the process for determining pH of tobacco products according to the Centers for Disease Control and Prevention (CDC) protocol for CDC and FDA reporting. This method was specifically developed for smokeless tobacco products, but has been shown to be fit for other tobacco products as discussed in the validation section.
2. The first time pH values are determined for a tobacco product, measure the pH at 5, 15, and 30 minutes. If there is less than a 10% change between the 5 and 30 minute pH values then subsequent replicate pH determinations will be made at 5 minutes after the addition of reagent water. If there is more than a 10% change between pH values over 30 minutes then pH will be measured at 15 minute intervals until the pH value is stable and does not change more than 10% over 15 minutes. The final pH value will be reported.
3. This procedure is applicable to samples ground to less than 4 mm particle size within the 4.01 to 10.01 pH range. Results are reported to two decimal places.

B. DEFINITIONS

1. pH - numerical expression of the hydronium ion concentration in an aqueous solution. Mathematically, pH is expressed as the – log $[H^+]$, where $[H^+]$ is the concentration of hydrogen ions in moles per liter.
2. Internal Quality Control Sample (IQCS) – A method process control sample (monitor sample) with an established target and control limits.

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 (i.e., moist snuff, dry snuff, snus, plug, twist, pellet, loose leaf chew, and portioned pouches of the aforementioned products. 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. audits of the procedure.

3. Tobacco products 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. This method has been shown to be fit for the determination of pH in ground tobacco, cigarette filler, and ground cigars[2].
5. (b) (4)

Grand Average	Std Deviation	% RSD
(b) (4)		

E. EQUIPMENT AND APPARATUS

1. Equipment and Apparatus Required
   a. pH meter, Orion 720A+, Orion Part Number 233706-A01, or equivalent.
   b. pH electrodes,
      1) Orion Ross™ combination pH probe, semi-micro electrode with BNC connector, part number 8103BN, Fisher Scientific, or equivalent.
      2) Orion Epoxy ATC Probe, part number 917005, Fisher Scientific, or equivalent.
   c. Extraction vessel: Beakers, polypropylene, 30-mL, FisherBrand™ part number FB012919.
   d. Analytical Balance, minimum of 100-gram capacity, resolution to 0.0001.
   e. Graduated cylinder, Certified, 25-mL capacity, Class A “To Deliver”.
   f. Nitrile Gloves, powder-free, disposable.
   g. Stir bar, PTFE (Teflon) coated, 20-mm x 3-mm, from Big Science, Inc. part number SBM-2003-MIC.
   h. Kimwipe KIMTECH™ wipers.
   i. NIST Traceable stopwatch/timer calibrated by an accredited calibration laboratory.
2. Instrument Maintenance
   a. Clean and/or replace probe as needed based on system performance, eg. if the pH for the monitor sample is below acceptable limits. Clean the probe per the Thermo Scientific Orion pH Electrode Cleaning Solutions instruction sheet[9,10].

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F. CHEMICALS AND REAGENTS

1. pH electrode storage solution, Fisher Scientific™ Orion™ Cat. No. 910007.
   a. This solution may also be made by the lab by adding 1 gram of KCl into 200 mL of certified pH 7 buffer.
2. pH standard buffer solutions
   a. pH 4.01 ± 0.01 buffer, e.g. Thermo Scientific™ Orion™ pH Buffer Individual Use Pouches, pack of 25, Cat. No. 910425.
   b. pH 7.00 ± 0.01 buffer, e.g. Thermo Scientific™ Orion™ pH Buffer Individual Use Pouches, pack of 25, Cat. No. 910725.
   c. pH 10.01 ± 0.02 buffer, e.g. Thermo Scientific™ Orion™ pH Buffer Individual Use Pouches, pack of 25, Cat. No. 911025-WA.
3. SPEX CertiPrep, Inc, certification: The buffers obtained from Thermo are sent to SPEX CertiPrep for certification. See work instruction WI 097-1005[3] for details.
4. Reagent water: complying with ASTM Type I of ISO 3696:1987[4], or better

G. SAMPLE REQUIREMENTS

1. pH determinations must be performed in a laboratory at a room temperature of 22°C to 25°C (71.6°F to 77.0°F) and should not vary more than 1°C (1.8°F) during analysis. Reagents and samples must be at a room temperature of 22°C to 25°C (71.6°F to 77.0°F).
2. The target tobacco weight for each replicate analysis of loose/ground tobacco is 2.00 g ± 0.02 g.
3. Number of sample replicates:
   a. CDC samples require two replicates and FDA samples require seven replicates, unless otherwise specified.
   b. Query the sample project code in LIMS to determine the number of sample replicates.

   Note: Consult laboratory management for instructions on preparing samples not described above.
4. Samples that require grinding and/or removal of filler from cigarettes or cigars are prepared according to instructions provided per form 099-1100 “AS sample preparation instruction sheet”, and WI 097-1108[5] “Sample Preparation”.
   a. Tobacco and cigarette filler is ground at ambient temperature.
   b. Chewing tobacco, cigar filler, and pipe tobacco are freeze ground.
   c. Pouched tobacco products:
      1) CDC reporting: Intact portions are freeze ground. The ground samples are thoroughly mixed to form a composite for aliquoting for analysis.

2) FDA reporting: Unit portions are cut in half. The tobacco and pouch are added directly to the extraction vessel for analysis.

d. (b) (4) Six (6) (b) (4) are broken into small pieces and added into the extraction vessel.

e. Monitor sample (IQCS): A monitor sample (such as CRP 3) is prepared as a method control with each batch of samples in order to monitor the performance of the method overtime.

Note: Consult laboratory management for instructions on preparing samples not described above.

H. PROCEDURE

1. Sample Handling

Note: Care should be taken in handling samples stored in the freezer / refrigerator. Sufficient time should be given to allow the sample material to reach room temperature before sample preparation.

a. Unopened IQCS: (stored at or below -20°C)

1) Remove an unopened can from the freezer and place the can in the refrigerator for 1-2 days. Remove the can from the refrigerator and allow the unopened can to equilibrate to ambient conditions on a lab bench for approximately 24 hours. Do not open the can during equilibration.

2) Open the can and transfer its contents to a 4 oz. Nalgene bottle (or equivalent). Label it with “Date Open”, “The type of the Monitor”, the expiration date and initials. The opened monitor expires two months after opening.

3) Close the bottle tightly and store it in a refrigerator.

4) For subsequent usage, allow the IQCS to come to room temperature (about 1 hour). Shake bottle to uniformly distribute the contents before analysis. Return the bottle to the refrigerator between uses.

b. Test Samples:

1) Shake bottle or gently mix with a spatula to ensure contents are homogenized before analysis.

2. Calibration

a. Set up and operate the pH meter and electrode according to the manufacturer’s instructions.

b. Calibrate the pH meter daily before use, with SPEX CertiPrep certified standard pH buffers 4.01, 7.00, and 10.01:

1) Pour pH buffers into clean, dry, separate extraction vessels and add a magnetic stir bar to each.

2) Remove the electrodes from the storage solution, rinse thoroughly with reagent water, and blot with a clean dry Kimwipe.
3) Place the extraction vessel containing pH buffer 4.01 on the magnetic stirrer to create a slight vortex and lower the electrodes into the pH buffer so that the bulb and liquid junction frit on the pH electrode and the temperature probe are covered by the solution. Allow the reading to stabilize and record the pH value before and after accepting the measurement.
4) Remove the electrode from the buffer solution and rinse thoroughly with reagent water, and blot with a clean Kimwipe.
5) Repeat steps H.2.b.3-4 for the pH buffers 7.00 and 10.01.
6) Ensure the electrode slope is within 92% to 102% before the electrode is used for sample analysis.
7) Record the calibration data in the pH calibration logbook.
8) Refer to the pH meter manual[8] or laboratory management, if the calibration is unacceptable. Perform and document any necessary corrective action.
9) Rinse the electrodes thoroughly with reagent water, blot with a Kimwipe and place the electrodes in the storage solution.

3. Analysis

Note: Use form 099-3104 “CDC pH worksheet” to record sample weights to the nearest 0.0001g, time of the addition of reagent water, pH measurements to the nearest 0.01 pH unit, and temperature measurements to the nearest 0.1°C. Ensure laboratory conditions meet the temperature requirement (between 22 ºC to 25 ºC or 71.6 ºF to 77.0 ºF) and record this information in the pH calibration logbook.

a. Calibration verification:
1) Pour the pH buffer 7.00 (QC sample) into a clean, dry extraction vessel and add a magnetic stir bar.

Note: Each QC sample is prepared separately.

2) Remove the electrodes from the storage solution, rinse thoroughly with reagent water, and blot with a clean dry Kimwipe.
3) Place the extraction vessel on the magnetic stirrer to create a slight vortex and lower the electrodes in QC sample so that the bulb and liquid junction frit on the pH electrode and the temperature probe are covered by the solution.
4) Record the pH and temperature measurements in the instrument logbook and CDC pH worksheet. Refer to the Quality Control and Acceptance Criteria section (H.5.) for additional information.

5) Rinse the electrodes thoroughly with reagent water, blot with a Kimwipe and either store the electrode in the storage solution or continue with analyses.

b. IQCS: Refer to the Procedure section (H.1.a.) for additional information.

1) Weigh 2.00 g ± 0.02 g into a tarred extraction vessel.

2) Record the weight to the nearest 0.0001 g.

Note: Each IQCS sample is prepared separately.

3) Add 20 mL reagent water to a 25-mL certified (TD) graduated cylinder and pour into the extraction vessel. Record the time the reagent water was added.

4) Place a stir bar into the extraction vessel.

5) Place the extraction vessel on a magnetic stirrer to create a slight vortex and stir continuously throughout testing.

6) Remove the pH and temperature electrodes from the storage solution, rinse thoroughly with reagent water, and blot with a Kimwipe.

7) Lower the electrodes into the extraction vessel so that the bulb and liquid junction frit on the pH electrode and the temperature probe are covered by the solution.

8) Record the pH and temperature measurements at the 5±1 minute time point, using a calibrated timer, while the sample is being stirred. Refer to the Quality Control and Acceptance Criteria section (H.5.a.) for additional information.

9) Rinse the electrodes thoroughly with reagent water and blot with a Kimwipe before measuring the pH and temperature of the next sample or place the electrodes in the storage solution.

c. Test Samples:

1) Loose/Ground tobacco/Ground Filler/Ground pouch samples:

a) Refer to the Sample Requirements section (G.3.) for the required number of sample replicates.

b) Weigh 2.00 g ± 0.02 g of the sample into the tarred extraction vessel.

c) Record the weight to the nearest 0.0001 g.

d) Add 20 mL of reagent water using a 25-mL certified (TD) graduated cylinder into the extraction vessel. Record the time the reagent water was added.

e) Proceed to step H.3.d. (Stirring and pH measurement) for pH measurement.

2) Intact pouch samples (typically for FDA testing):
   a) Refer to the Sample Requirements section (G.3.) for the required number of sample replicates.
   b) Weigh a sufficient quantity of unit portions (tobacco with pouch), into the tared extraction vessel, to achieve a weight between 0.80 g - 2.50 g.
   c) Cut the pouch(es) in half with clean scissors and add the tobacco and pouch directly to the tared extraction vessel.

      Note: The pouch paper can be further cut into smaller pieces to facilitate stirring.
   d) Record the weight to the nearest 0.0001 g.
   e) Add 20 mL of reagent water using a certified (TD) 25-mL graduated cylinder into the extraction vessel. Record the time the reagent water was added.
   f) Proceed to step H.3.d. (Stirring and pH measurement) for pH measurement.

3) (b) (4)® samples:
   a) Refer to the Sample Requirements section (G.3.) for the required number of sample replicates.
   b) Break Six (6) (b) for each sample replicate into the tarred extraction vessel.
   c) Record the weight to the nearest 0.0001 g.

      Note: The weight may exceed the target weight of 2.00g ± 0.02 g.
   d) Add reagent water, at a 1:10 w/v ratio, using a certified (TD) 25-mL graduated cylinder into the extraction vessel. Record the time the reagent water was added.

      Example: If the sample weight is 2.2 g, then add 22 mL reagent water.
   e) Proceed to step H.3.d. (Stirring and pH measurement) for pH measurement.

d. Stirring and pH measurement
   1) Place a stir bar into the extraction vessel.
   2) Place the extraction vessel on a magnetic stirrer to create a slight vortex and stir the sample continuously throughout analysis.
   3) Remove the pH and temperature electrodes from the storage solution, rinse thoroughly with reagent water, and blot with a clean Kimwipe.

4) Lower the electrodes into the extraction vessel so that the bulb and liquid junction frit on the pH electrode and the temperature probe are covered by the solution.
5) Record the pH value and temperature at 5±1, 15±1 and 30±1 minutes, using a calibrated timer, from addition of the reagent water.

   Note: If there is less than a 10% change between the 5 and 30 minute pH values then subsequent replicate pH determinations can be made at 5 minutes after the addition of reagent water. Refer to the Calculations section (H.4.b) for the percent change formula.

   Note: If there is more than a 10% change between pH values over 30 minutes then continue to measure the pH of the sample at 15 minute intervals until the pH value is stable and does not change more than 10% over 15 minutes.
6) Perform subsequent sample replicate pH determinations based on the time to stabilize the pH of the first replicate.

   Note: The first time pH values are determined for a tobacco product, the pH must be measured at multiple time points, as described above to determine when there is less than a 10% change between subsequent time points. Unless documented for a particular product/brand, a full 30 minute time study must be conducted first to establish the analysis time for subsequent replicates of the same matrix. Any product change will require that the full time study be conducted.
7) Record the pH value and temperature.
8) Remove the electrodes from the sample and rinse the pH electrode and temperature probe thoroughly with reagent water and place into the next sample or into storage solution.

   Note: The meter may automatically display HOLD (displayed as HLD) after a period of inactivity, and it will be necessary to press the measure key in order to take additional readings.
9) Continue sample measurements to complete the batch.

   Note: A maximum of ten (10) pH determinations must be bracketed by an IQCS.

e. IQCS and QC sample measurements:
   1) Record the pH value and temperature.
   2) Refer to the Quality Control and Acceptance Criteria section (H.5.) for additional information.
f. Enter results in LIMS and prepare a regulatory packet for review and approval by authorized personnel.

g. After the regulatory packet has been approved, report, authorize and store data in accordance with SOP 095-0037[6], Results Verification and Reporting.

4. Calculations and Reporting
   a. The final pH result is the average of replicate pH values.
   b. Percent change is calculated using the sample replicate pH value with time.

$$\text{Percent Change} = \left(\frac{(T_x \text{ value} - T_R \text{ value})}{T_R \text{ value}}\right) \times 100\%$$

Where:

T_x Value: = the pH value from the 30 minute measurement after the addition of reagent water

T_R Value = the pH value from the 5 minute measurement

5. Quality Control and Acceptance Criteria
   a. A new IQCS sample must be run every 10 determinations.
      1) Plot the mean of the pH values for the IQCS monitor(s) on the QI Macros control chart. The rules and information on how to address out-of-control conditions are documented on the control charts. All samples must be bracketed by passing IQCS samples.

I. REFERENCES

1. 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.
2. CORESTA Tobacco and Tobacco Products Analytes Technical Report: 2017 TSNA, pH, and Moisture (Oven Volatiles) Collaborative Study, July 2017.
3. WI 097-1005, pH Buffer Order and Certification Process for Physical Properties Lab, April 27, 2017.
4. ISO 3696:1987 Water for analytical laboratory use – specification and test methods.
5. WI 097-1108, Sample Preparation, April 4, 2017.
6. SOP 095-0037, Results Verification and Reporting, November 1, 2015.
7. “pH 7 calibration verification sample acceptance range,” Excel Report, April 18, 2012, C. T. Connell
8. Orion 720A+ Bench top pH and pH/ISE Meter Instrument Manual from Thermo Electron Corporation; 2003, Revision C.
9. Orion Ross™ pH Electrode Instrument Manual; 2003, Revision C.

10. Thermo Scientific Orion pH Electrode Cleaning Solutions, 254789-001, Rev. A 0807.

J. FORMS

1. 099-3104, CDC pH Worksheet
2. 099-1100, AS Preparation Instruction Sheet