



Altria

Altria Client Services

Analytical Sciences
Policies
Procedures
Instructions

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pH Analysis of Tobacco - Lancaster

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NOTICE TO USERS

This method may involve the use of hazardous substances and/or equipment. The user must not assume that all of the safety issues associated with its use have been described. Prior to use of this method, the user is responsible for establishing appropriate safety and health practices and determining the applicability of regulatory requirements.

The person performing this method must be trained according to the safety guideline specific to the job task and area of assignment. The person must use all appropriate safety equipment referenced by the facility's safety guidelines. Copies of Material Safety Data Sheets (MSDS) are available from the ALCS Intranet Safety web site, Facility Safety Department or the area supervisor.

A. Purpose and Scope

1. This method describes the procedure for measuring pH of smokeless tobacco aqueous extract (5g/100 mL nominal.) It is written for employees of Lancaster Professional Scientific Staffing. The pH calibration range is from pH 4 to 10; pH is reported to the nearest 0.01 pH unit.
 - a. The pH calibration range is from pH 4 to 10; pH is reported to the nearest 0.01 pH unit.

B. Definitions

1. **pH** — A numerical expression of the hydrogen, or 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.

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 the designated subject matter expert.
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. Not applicable

E. Equipment Requirements, Apparatus and Reagents

1. **Equipment Requirements and Apparatus**
 - a. pH meter meeting the following specifications:
 - (1) Resolution: 0.001 pH
 - (2) Relative Accuracy: ± 0.005 pH
 - (3) Automatic Temperature Control (ATC) capability and an ATC probe or an electrode with a built-in ATC sensor.
 - (4) pH meters meeting these criteria include:
 - (a) Mettler Toledo Seven-Multi S47 Dual-Channel pH/Conductivity Meter (p/n 01-913-804.
 - (b) Metrohm 855 Robotic Titrator with 59 position carousel
 - i. Tiamo Full version 2.2 (or later) pH Software; .

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- (5) Optional:
 - (a) Automatic buffer recognition is preferred.
 - (b) An RS232 output (for connection to pc or LIMS systems).
 - (c) Conductivity module is not required for this method.
- b. pH Electrode. Examples of acceptable electrodes include:
 - (1) Thermo Orion ROSS Sure-Flow, 8272BN (preferred) or 8172N
 - (a) BNC to Metrohm F-Plug, optional conversion cable (for use with Metrohm Auto-pH unit); Metrohm p/n: 020977663.
 - (2) Metrohm Unitrode (electrode with PT1000 probe), model 6.0258.010, Metrohm p/n 020910682.
 - (a) Alternately, Metrohm electrode body and cable may be purchased separately:
 - i. Unitrode electrode body, p/n: 0209110691.
 - ii. Unitrode cable, p/n: 020910708.
- c. PT1000 Temperature Probe. Probe may be separate or incorporated into the electrode body as a combination electrode. Examples of separate acceptable PT1000 probe:
 - (1) Mettler Toledo PT1000 (p/n 51300165)
- d. Balance, top-loading, having 0.01 g resolution or better.
- e. Magnetic stirrer(s), capable of maintaining a stir rate of up to ~600 rpm
- f. Griffin-type beaker or Erlenmeyer flask, polypropylene or glass, with the capacity to match sample size and extraction volume. (e.g., For 100 mL extraction volume, preferred capacity is 150 mL. Possible vessel sources include Fisher-brand Polypropylene Disposable Beakers, 30 mL (Cat. No. 01-291-9) and 150 mL (Cat. No. 01-291-11.)
- g. Stir Bars, various sizes to match extraction volume. For 100 mL extraction volume, use Fisher-brand Polygon Stir Bars, $\frac{3}{8}$ " Diam. X 1" Length (Cat. No. 14-512-125). For reduced extraction volumes use Fisher-brand Magnetic Micro stir bars, $\frac{3}{8}$ " Diam. X $\frac{1}{2}$ " Length (Cat. No. 14-513-98.)
- h. Sample cups (for use with Metrohm 855 Autosampler):
 - (1) Option 1: Beaker, straight-walled, polypropylene, 60 mL capacity, Metrohm p/n 020292184 (pack of 500).
 - (2) Option 2: Beaker, straight-walled, polypropylene, 120 mL capacity, Metrohm p/n 020212250 (pack of 100).
- i. Optional Equipment for use with Mettler Seven-Multi pH unit:
 - (1) pH Software, WinWedge (Standard Addition) or Mettler Toledo LabX (for capturing pH data).

2. Chemicals and Reagents

- a. Electrode Storage Solution:
 - (1) For Metrohm Unitrode, use Metrohm Cat. No. 020092600.
 - (2) For Ross pH Electrode, use Thermo Fisher Cat. No. 810001.

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- b. Electrode Filling Solution (3M KCl), Metrohm Cat. No. 020096055 or equivalent.
- c. NIST Traceable, pH Buffers (Calibration Standard Solutions), or equivalent as determined by accreditation, manufacturer's pH specification range, and NIST traceability. ISO 17025 / Guide 34 accredited standards are preferred.
 - (1) Thermo Scientific Orion Application Solutions
 - (a) pH 4.01 buffer, p/n 910104.
 - (b) pH 7.00 buffer, p/n 910107.
 - (c) pH 10.01 buffer, p/n 910110
 - (2) Intermediate Quality Control Buffer Solutions
 - (a) Fisher pH 5.00, Cat. No. SB102-500 for 500 mL.
 - (b) Fisher pH 8.00, Cat. No. SB112-500 for 500 mL

Note: Alternate QC buffers may be used to be closer to the sample pH.
- d. Water, distilled or deionized.
- e. 2-Propanol, ACS Reagent Grade, or equivalent (for electrode washing).

F. Environmental and Sample Requirements

1. Environmental Requirements

- a. Not applicable

2. Sample Requirements

- a. Avoid direct skin contact with samples – wear nitrile gloves during preparation and analysis.
- b. Determine the appropriate sample weight based on all requested testing requirements.
- c. For loose tobacco, blend the sample prior to weighing
- d. Sample extract pH is stable for up to 4 hours at 20-25°C if covered.

G. Reagent and Standard Preparation

- 1. Not applicable

H. Equipment Setup

NOTICE

Inspect the power cord on the instrument for frayed or damaged wires and broken plugs. Have defective wires or plugs replaced or repaired by qualified personnel.

Check the power supply requirements against the name plate on the instrument. If there is any doubt about the power supply, plugs or wiring, contact the facility electrician for assistance.

Plug the instrument power cord into the receptacle.

- 1. Program the pH meter setup parameters.
 - a. Metrohm 855 Autosampler Measuring Parameters:
 - (1) Signal Drift: 0.5 mV/min

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- (2) Min. Waiting Time: 30 seconds
- (3) Max. Waiting Time: 180 seconds
- (4) Measuring Interval: 2.0 seconds
- (5) Stop Measured Value pH: Off
- (6) Temperature: 25.0°C
- (7) Calibration Mode: Linear (default)
- (8) Customized pH Buffer Group: Enter values according to the certified values for the lot as defined on the Certificate of Analysis. See Table 1 for typical values for the specified Thermo Orion product lots.

Table 1: Customized pH Buffer Settings

| Temp (°C) | Buffer 1 | Buffer 2 | Buffer 3 |
|--------------|---------------------|---------------------|----------------------|
| | Lot OR1 | Lot OR1 | Lot OR1 |
| | <i>Nominal pH 4</i> | <i>Nominal pH 7</i> | <i>Nominal pH 10</i> |
| 10.0 | 4.00 | 7.06 | 10.18 |
| 20.0 | 4.00 | 7.01 | 10.06 |
| 25.0 | 4.01 | 7.00 | 10.01 |
| 30.0 | 4.02 | 6.98 | 9.97 |

Note: Review the certified buffer tables for each new lot of reference buffers received, and adjust the custom buffer template accordingly.

b. Mettler Seven-Multi Parameters:

- (1) pH Meter Resolution: High resolution (for reading to three decimal places)
- (2) Stability: Normal
- (3) Endpoint Selection: Auto
- (4) Alarm Limits Function: Off
- (5) Calibration Mode: Segmented
- (6) pH Buffer Mode: Customized Buffer Group. If using Thermo Orion buffers, enter values according to Table 2.

Table 2: Customized pH Buffer Settings

| Temp (°C) | Buffer 1 | Buffer 2 | Buffer 3 |
|-----------|---------------------|---------------------|----------------------|
| | <i>Nominal pH 4</i> | <i>Nominal pH 7</i> | <i>Nominal pH 10</i> |
| | | | |
| 10.0 | 4.000 | 7.060 | 10.180 |
| 20.0 | 4.000 | 7.010 | 10.060 |
| 30.0 | 4.020 | 6.980 | 9.970 |

I. Calibration

1. Flush the electrode junction with at least 1-2 mL of filling solution prior to calibration.
2. Fill the electrode chamber with filling solution and remove any air bubbles by gently shaking or tapping the electrode.

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3. Re-equilibrate the electrode in electrode storage solution for at least five minutes before proceeding.
4. If using LabX Software, Setup the Instrument Configuration according to Attachment 1 (Optional)
5. Calibrate the pH meter and electrode
 - a. Calibrate the meter prior to analyzing samples using buffers for pH 4, 7, and 10 by following the instructions in the meter's user manual

Note: Subsequent calibration(s) should be performed, if necessary, after failed Quality Control Samples (See Section J.1., "Analyze the Quality Control buffer").

- (1) Add each buffer to a clean, dry beaker containing a stir bar that will provide a slight vortex while mixing. Do not allow the stir bar to contact the electrode or damage will result.
- (2) Stir the buffer while reading the pH value. Set the speed of the stir plate to achieve a gentle vortex.
- (3) While measuring the buffer, confirm that the internal filling solution is at least one inch above the solution to be measured and the Sure-Flow Junction (reference junction) is covered by the solution.
- (4) Verify the quality of the calibration using the criteria in Table 3.

Table 3: pH Meter Calibration Criteria

| pH Meter Type | Calibration Slope | pH 7 Buffer | |
|----------------------------------|-------------------|-------------|---------------|
| | | Offset | pH(0) |
| Metrohm Robotic 855 Titrosampler | 95 – 102 % | ± 15 mV | 6.493 – 7.507 |
| Mettler Seven-Multi | 95 – 105 % | ± 15 mV | |

Note: If any criterion is outside of these ranges, do not proceed with sample analysis. Consult the meter's User Manual, Thermo Customer Support, and/or departmental technical leadership for troubleshooting.

- (5) Record the calibration results and the pH buffer lots used in the pH meter logbook.

J. Test Procedure

1. Analyze the Quality Control (QC) buffer.
 - a. Check the meter's calibration by measuring the pH of a NIST-traceable buffer in the expected pH range of the sample (e.g., Fisher pH 8 buffer).
 - b. Record the lot of buffer solution used and the pH value.
 - c. Add the buffer to a beaker, add a stir bar large enough to achieve a slight vortex (if the pH meter does not use a propeller to stir the sample).
 - d. Place the electrode in the buffer, submerging the Sure-Flow Junction (reference junction) below the level of the solution.
 - e. Allow the electrode to equilibrate for a minimum of 30 sec. prior to reading it.
 - f. Read the pH once the reading has stabilized.
 - a. For Mettler units, Press the "Read" button on the pH meter.

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- b. Record the pH when the Ready symbol appears. (If the meter is connected to a PC with Mettler LabX pH software, the measurement is captured by the software when the Ready symbol appears.)
 - c. If the pH value is within ± 0.04 of its nominal value at the prescribed temperature, continue with step J.1.g.
 - d. If this buffer does not read within ± 0.04 of its nominal value at the prescribed temperature, refer to Section K., "Quality Control", then recalibrate.
 - e. After recalibration, if the QC buffer is within acceptable limits, repeat any samples measurements that were not bracketed by acceptable QC buffer measurements.
 - g. If the QC buffer value is not within the acceptance limits after performing an additional calibration, do not proceed with sample analysis and begin troubleshooting. Do not resume sample analysis until the problem is resolved.
 - h. Measure the pH of the QC buffer after every tenth sample and after the last sample of the sample set.
 - a. The same aliquot of QC buffer may be used throughout the same day as long as it continues to meet the acceptance criteria of. (± 0.04 pH units from certified value). Covering the buffer solution between measurements is recommended to prevent buffer instability during testing.
2. Analyze the test Sample:
 - a. Collect distilled or Milli-Q Water for the next day's extractions into a sealable vessel and allow it to sit sealed overnight to reach room temperature. Fill the vessel so that there is minimal headspace.
 - b. Transfer 5.00 ± 0.05 g of sample to an extraction beaker (or cup), recording the weight to the nearest 0.01 g. Retain enough sample to repeat the pH analysis if necessary.

Note: If insufficient sample is provided to allow for a 5.00 g sample size or complete a duplicate analysis, sample weight may be reduced along with the extraction volume to maintain the same sample: water ratio.

 - c. Add water to the sample to achieve a 5 g / 100 mL water (1:20) ratio.
 - d. Add a stir bar large enough to achieve good sample agitation.
 - e. Cover the sample and place it on a non-mechanical magnetic stirrer.
 - f. Stir the sample for 60 minutes with good sample agitation.
 - (1) For Example, with MST:
 - (a) Fine Cut: ~ 400 rpm
 - (b) Long Cut: ~ 600 rpm
 - g. Measure the sample pH within four hours of extraction. (If using a pH meter equipped with a propeller, remove the stir bar prior to pH measurement.)

Note: Sample extract pH is stable for up to 4 hours at 20-25°C if covered.

 - h. When taking the measurement of a sample, stir the sample at a rate that achieves a gentle vortex
 - i. Record the pH to the nearest 0.01 pH units when the Ready symbol appears. If the meter is connected to a PC with pH software, the measurement is captured by the software once the pH stabilizes.

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- j. Rinse the electrode with distilled or Milli-Q water, then blot the electrode to remove excess water between samples and upon completion of the sample set.

Note: If using an automated pH system, a rinse with approximately 20-25 % 2-propanol in DI water is recommended immediately following pH measurement. This rinse may be followed by a DI water rinse to ensure sufficient electrode hydration.

K. Quality Control

1. Meter Performance

- a. Once a week, change the readout mode of the meter to millivolts and read the 4, 7, and 10 buffer solutions used during meter calibration.
- b. Confirm a reading of 0 ± 15 mV for the pH 7 buffer.
- c. Criteria for the pH 4 and 10 buffers:
 - (1) pH 4 buffer: 160 – 180 mV less than the pH 7 measurement
 - (2) pH 10 buffer: 160 – 180 mV greater than the pH 7 measurement
- d. Record each reading in the pH meter logbook.
- e. If these criteria are not met, refer to the meter's user manual and do not analyze test samples until the problem is resolved

2. Electrode Performance

- a. Maximum response time (for sample measurement):
 - (1) Mettler: 60 seconds
 - (2) Metrohm: 180 seconds
- b. Drift
 - (1) Mettler: 0.003 pH units (30 second span)
 - (2) Metrohm: 0.5 mV/minute

3. Sample Set Acceptance Criteria

- a. Sample readings are acceptable if they are preceded by a passing calibration and bracketed by Quality Control Samples meeting the acceptance criteria of ± 0.04 pH units at the specified temperature.
- b. Repeat the analysis of any samples not meeting these criteria.
- c. If part of a sample set meets these criteria while the rest does not, the former is acceptable, but the latter must be repeated.
- d. If a Quality Control sample fails in the middle of a sample set without an assignable cause, recalibrate the meter according to Section I. and do not resume analyzing samples until the meter is confirmed to be working properly again

L. Calculations

1. Not applicable – pH value is read directly from the pH meter display

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M. Technical Information

1. Follow the instructions in the Electrode's user manual for installation, use, maintenance, and troubleshooting information.
2. When not in use, store electrodes as recommended by their user manuals
3. When in use, fill the electrode with enough filling solution to cover the internal glass coil.
4. While performing a calibration or measuring quality control or test samples, ensure that the filling hole is open.
5. Avoid storing pH Buffer Solutions, Milli-Q Water used for sample extractions, or samples in direct exposure to air conditioning vents.
6. Avoid working in a laboratory with temperature fluctuations.

N. Records Requirements

1. All records generated by this procedure should be handled and stored in accordance with the Altria Records Management Policy.
2. pH Meter Logbook
3. pH Meter Calibration Worksheet

O. References

1. Not applicable

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P. Appendices

Attachment 1 LabX Instrument Configuration

| | |
|------------|---------------|
| Instrument | 7 Multi V.2.X |
| Com Port | 1 |
| Baud Rate | 9600 |
| Parity | None |
| Data Bits | 8 |
| Stop Bits | 1 |
| Hand Shake | 0-None |