WHO TobLabNet
Official Method
SOP 14

Standard Operating Procedure for Determination of the pH of Smokeless Tobacco Products

No Tobacco Unit (Tobacco Free Initiative)
Tobacco Laboratory Network (TobLabNet)

World Health Organization

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No.: SOP 14
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World Health Organization

World Health Organization Tobacco Laboratory Network

Standard operating procedure for

Determination of the pH of smokeless tobacco products

Method:	Determination of the pH of smokeless tobacco products
Analytes:	pH
Matrix:	Smokeless tobacco products
Last update:	December 2021

No.: SOP 14
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FOREWORD

Smokeless tobacco products are gradually attracting the interest of public health organizations. A request was made by the WHO Framework Convention on Tobacco Control (WHO FCTC) Conference of the Parties (COP) at its fifth session (Seoul, 2012) to identify options to regulate chemicals in smokeless tobacco products. This document is prepared in response to the request made by the COP at its seventh session (Delhi, 2016) to the WHO FCTC Secretariat to invite WHO to finalize the standard operating procedures (SOPs) for measuring nicotine and tobacco-specific nitrosamines as requested by decision FCTC/COP6(12) 2b.ii. In pursuance of this request, WHO organized a collaborative study involving its Tobacco Laboratory Network (TobLabNet) testing laboratories, which tested materials for which there was some chemical characterization, represented a range of common forms of smokeless tobacco products and differed in physical and chemical properties. The assessment of applicability and adaptability of validated WHO SOPs to smokeless tobacco products and the recommended methods are presented in this SOP.

This document was prepared by members of the WHO TobLabNet as an analytical method SOP for measuring the pH of smokeless tobacco products. pH is one of the key influences on the nicotine delivery capacity of a product.

INTRODUCTION

In order to establish comparable measurements for testing tobacco products globally, consensus methods are required for measuring specific parameters of smokeless tobacco products. The WHO TobLabNet reviewed commonly used procedures for determining the pH of smokeless tobacco products in order to prepare a procedure as a WHO TobLabNet SOP.

This SOP was adapted from CORESTA Recommended Method No. 69 [**2.1**] to describe the procedure for determination of the pH of smokeless tobacco products.

1. SCOPE

This SOP gives general guidelines for measurement of the pH of smokeless tobacco products by water extraction followed by pH meter readings. This method is suitable for measuring pH in the range 4–14 for smokeless tobacco products.

2. REFERENCES

2.1 CORESTA Recommended Method No. 69 Determination of pH of Tobacco and Tobacco Products

2.2 ISO 3696:1987 Water for analytical laboratory use – Specification and test methods.

2.3 United Nations Office on Drugs and Crime. Guidelines on representative drug sampling. Vienna, Laboratory and Scientific Section, 2009 (http://www.unodc.org/documents/scientific/Drug_Sampling.pdf).

2.4 World Health Organization. Standard operating procedure for validation of analytical methods of tobacco product contents and emissions. Geneva, Tobacco Laboratory Network, 2017 (WHO TobLabNet SOP 02).

2.5 ISO 5725-1. Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

2.6 ISO 5725-2: Accuracy (trueness and precision) or measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

3 TERMS AND DEFINITIONS

3.1 *pH*: pH in smokeless tobacco products

3.2 *Smokeless tobacco*: Tobacco-containing part of a smokeless tobacco product.

3.3 *Smokeless tobacco products*: Products entirely or partly made of leaf tobacco as the raw material that are manufactured to be used by sucking, chewing or snuffing (Article 1(f) of the WHO FCTC), including snus (dry and wet), chewing tobacco or a mixture of material originating from tobacco plants.

3.4 *Laboratory sample*: Sample intended for testing in a laboratory, consisting of a single type of product delivered to the laboratory at one time or within a specified period.

3.5 *Test sample*: Product to be tested, taken at random from the laboratory sample. The number of products taken shall be representative of the laboratory sample.

3.6 *Test portion:* Random portion from the test sample to be used for a single determination. The number of products taken shall be representative of the test sample.

4. METHOD SUMMARY

Aqueous extracts of smokeless tobacco product samples are prepared, and their pH measured with a pH electrode.

5. SAFETY AND ENVIRONMENTAL PRECAUTIONS

5.1 Take routine safety and environmental precautions, as in any chemical laboratory activity.

5.2 The testing and evaluation of certain products with this test method may require the use of materials or equipment that could be hazardous or

harmful to the environment; this document does not purport to address all the safety aspects associated with its use. All persons using this method have the responsibility to consult with the appropriate authorities and to establish health and safety practices as well as environmental precautions in conjunction with any existing applicable regulatory requirements prior to its use.

5.3 Special care should be taken to avoid inhalation or dermal exposure to harmful chemicals. Use a chemical fume hood, and wear an appropriate laboratory coat, gloves and safety goggles when preparing or handling undiluted materials, standard solutions, extraction solutions or collected samples.

6. APPARATUS AND EQUIPMENT

Usual laboratory apparatus, in particular:

6.1 pH meter.

6.2 Shaker (linear type) configured to hold the extraction vessels in position.

6.3 Extraction vessels: Erlenmeyer flasks (100-mL) with stoppers, 100-mL Pyrex bottles with crimp seals and septa, 100-mL culture tubes with Teflon-lined caps or other suitable flasks.

7. REAGENTS AND SUPPLIES

All reagents shall be of at least analytical reagent grade unless otherwise noted. When possible, reagents are identified by their Chemical Abstract Service (CAS) registry numbers.

7.1 Water, compliant with grade 2 of ISO 3696:1987 [**2.2**], or better.

7.2 Standard pH buffer solutions (pH = 4.01, 9.21 or > 10 according to the availability of pH calibration solutions in the laboratory).

8. PREPARATION OF GLASSWARE

8.1 Clean and dry glassware in a manner to avoid contamination.

9. PREPARATION OF SOLUTIONS

Not applicable

10. PREPARATION OF STANDARDS

Not applicable

11. SAMPLING

11.1 Sample smokeless tobacco products according to the laboratory sampling procedure. Alternative approaches may be used to obtain a representative laboratory sample in accordance with individual laboratory practice or when required by specific regulation or availability of samples.

11.2 Constitution of test sample

11.2.1 Divide the laboratory sample into separate units (e.g., packet, container), if applicable.

11.2.2 Take an equal amount of products for each test sample from at least √n[**2.3**] of the individual units (e.g., packet, container).

12. PRODUCT PREPARATION

12.1 Remove the smokeless tobacco product from the packs or container. Include quality control samples (when applicable).

12.2 Take an appropriate, representative portion of the smokeless tobacco product according to individual laboratory practice (e.g., food analysis sampling approach may be applied).

12.3 Extract the smokeless tobacco from the smokeless tobacco product.

12.4 Combine and mix sufficient smokeless tobacco product samples to constitute about 2–5 g of homogeneous smokeless tobacco for each test sample.

13. PREPARATION OF THE SMOKING MACHINE

Not applicable

14. SAMPLE GENERATION

Not applicable

15. SAMPLE PREPARATION

15.1 Weigh at least 1.0 g ± 0.1 g of smokeless tobacco sample into a 100-mL Erlenmeyer flask. A sample weight range of 1–2 g is suggested for this method.

15.2 Add 20.0 mL ± 0.5 mL of water into the flask, and shake gently for about 30 min.

16. SAMPLE ANALYSIS

16.1 Calibration of pH meter

16.1.1 Calibrate the pH electrode with at least two pH buffer solutions (pH = 4.01 and 9.21 or above); produce a two-point calibration within the calibration range. Calibration and measurement of samples are performed consecutively, as the two must be completed at the same temperature.

16.1.2 The electrode slope should be within 95–105% of the calculated value before the electrode can be used for sample measurement.

16.1.3 Rinse the electrode with distilled water before and after each measurement.

16.2 pH measurements of samples

16.2.1 Measure the pH of the smokeless tobacco samples within 60 min of stopping shaking.

16.2.2 After shaking, let the sample flask stand for another 20 min to allow the solution to settle, filter through Whatman paper No 41, or centrifuge at medium speed at 10 000 rpm if necessary.

16.2.3 Record the temperature. All pH measurements should be performed at room temperature, 20–25 °C ± 1° C.

16.2.4 Measure the pH to two decimal places.

16.2.5 The pH of a sample must be measured repeatedly at 15-min intervals. The average measurement for 1 h (four readings) is reported. The maximum tolerated variation in pH between readings is 0.3 units.

16.2.6 Rinse the electrode with distilled water before and after each measurement.

17. DATA ANALYSIS AND CALCULATIONS

Not applicable

18. SPECIAL PRECAUTIONS

Not applicable

19. DATA REPORTING

19.1 The test report should provide the pH results to two decimal places

19.2 For more information, see WHO TobLabNet SOP 02 [**2.4**].

20. QUALITY CONTROL

20.1 Control parameters

Note: If the control measurements are outside the tolerance limits of the expected values, appropriate investigation and action must be taken.

Note: Additional laboratory quality assurance procedures should be carried out if necessary, in order to comply with the policies of individual laboratories.

20.2 Quality control sample

To verify the consistency of the entire analytical process, analyse a reference tobacco product, such as CORESTA Reference Products (CRPs), when available, in accordance with the practices of individual laboratories.

21. METHOD PERFORMANCE SPECIFICATIONS

Recovery of reference material is a surrogate measure of accuracy. Recovery is determined by measuring the level of moisture in reference smokeless tobacco products. The recovery is calculated from the following equation.

$$\text{Recovery (\%)} = 100 \times (\text{analytical result} / \text{certified value})$$

Table 1. Mean and recovery of pH in smokeless tobacco products

Smokeless tobacco sample	Certified value	Mean pH	Recovery (%)
CRP 1	8.4	7.68	92.8
CRP 2	7.7	8.05	103.6
CRP 3	7.1	7.04	99.5
CRP 4	6.2	5.95	95.7

22. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study conducted between September 2020 and March 2021, involving 13 laboratories and four samples (four CRP smokeless tobacco products), performed according to WHO TobLabNet Method Validation Protocol and this SOP, gave the following values for this method.

The test results were analysed statistically in accordance with ISO 5725-1 [**2.5**] and ISO 5725-2 [**2.6**] to give the precision data shown in Table 2.

Table 2. Precision limits for determination of pH in smokeless tobacco products

Reference tobacco product	N	Mean	Repeatability limit (r)	Reproducibility limit (R)
CRP1.1	13	7.68	0.17	1.70
CRP2.1	13	8.05	0.20	1.31
CRP3.1	13	7.04	0.10	2.84
CRP4.1	13	5.95	0.10	1.00

This document was prepared by the No Tobacco Unit of the Health Promotion Department of the World Health Organization (WHO) and members of the WHO Tobacco Laboratory Network (TobLabNet) as an analytical method standard operating procedure (SOP) for measuring the pH of smokeless tobacco products.

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