

WHO TobLabNet  
Official Method  
**SOP 13**

# **STANDARD OPERATING PROCEDURE FOR DETERMINATION OF MOISTURE CONTENT IN SMOKELESS TOBACCO PRODUCTS**

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



**World Health  
Organization**





**WHO TobLabNet  
Official Method  
SOP 13**

**Standard operating procedure for  
determination of moisture content in  
smokeless tobacco products**



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**No.:** SOP 13

**Date:** December 2021



**World Health  
Organization**

**World Health Organization  
Tobacco Laboratory Network**

**Standard operating procedure for**

**Determination of moisture content in  
smokeless tobacco products**

<b>Method:</b>	Determination of moisture content in smokeless tobacco products
<b>Matrix:</b>	Smokeless tobacco products
<b>Last update:</b>	December 2021





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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 some chemical characterization was available, represented a range of common forms of smokeless tobacco product 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 moisture content of smokeless tobacco products. Moisture content is one of the key influences on nicotine delivery by a product.

## INTRODUCTION

In order to establish comparable measurements of the moisture content of smokeless tobacco products and to prepare a procedure for WHO TobLabNet products globally, consensus methods are required to measure specific parameters in smokeless tobacco products. The WHO TobLabNet reviewed commonly used procedures in developing this SOP, such as CORESTA Recommended Method No. 76 [2.1] and AOAC Official method 966.02 [2.2].

### 1. SCOPE

This method specifies an oven-drying method for determining the moisture content of smokeless tobacco products. Moisture content (oven volatiles) is the reduction of the mass on drying a sample in a forced draft oven at a temperature regulated at  $100\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for  $3\text{ h} \pm 0.5\text{ min}$ . This method allows measurement of the volatile constituents of smokeless tobacco products, including water and flavouring components that are lost under the specified conditions. Accurate determination of moisture content in smokeless tobacco products is critical, as moisture content affects product stability and product integrity.

### 2. REFERENCES

- 2.1** CORESTA Recommended Method No. 76 Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products



- 2.2 AOAC Official Method 966.02 Loss on Drying (Moisture) in Tobacco Gravimetric Method
- 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](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, (WHO TobLabNet SOP 02).
- 2.5 ISO5725-1. 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 *Moisture content*: Moisture content in smokeless tobacco products, expressed as milligrams per gram.
- 3.2 *Smokeless tobacco*: Tobacco-containing part of a smokeless tobacco product.
- 3.3 *Smokeless tobacco products*: Products made entirely or partly of leaf tobacco as the raw material that are manufactured to be used by smoking, 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 a tobacco plant.
- 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.
- 3.7 *Wet smokeless tobacco weight*: Weight of the smokeless tobacco product before placement in the oven for testing its moisture content.
- 3.8 *Dry smokeless tobacco weight*: Weight of the smokeless tobacco product after placement in the oven for testing its moisture content.





#### **4. METHOD SUMMARY**

- 4.1** Moisture content is defined in this method as the reduction in mass when a sample is dried in an air draft oven at a temperature of  $100\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for  $3\text{ h} \pm 0.5\text{ min}$ .
- 4.2** After drying, the samples are cooled in a desiccator to room temperature to prevent uptake of humidity from the air.

#### **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 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** Air draft oven capable of maintaining the air temperature at  $100\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ .
- 6.2** Desiccator.
- 6.3** Analytical balance.
- 6.4** Crucible or evaporating dish to contain the samples, or equivalent.

#### **7. REAGENTS AND SUPPLIES**

- 7.1** Ultrapure water.

#### **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  $\sqrt{n}$ [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 0.5–2 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** Oven preparation



Turn on the oven, and set the temperature to 100 °C ± 1 °C. Allow the oven to equilibrate for at least 1 h before use, and ensure that the temperature stabilizes at 100 °C ± 1 °C.

## 15.2 Preparation of samples

**15.2.1** Weigh a clean, dry evaporating dish on an analytical balance, and record the weight as  $W_T$ .

**15.2.2** Remove the dish from the balance, and fill it with 1–5 g of the smokeless tobacco test sample, depending on the size.

**15.2.3** Place the dish with sample on the balance, and record the weight at  $W_1$  to 0.01-g accuracy.

**15.2.4** Check that the oven temperature is at 100 °C ± 1 °C, and place the samples in the oven.

**15.2.5** Close the oven door, record the start time, and leave for 3 hours ± 0.5 min.

**15.2.6** Repeat steps **15.2.1–15.2.5** for all samples.

## 16. SAMPLE ANALYSIS

**16.1** After 3 h ± 0.5 min, remove the evaporating dishes containing the samples from the oven, and place them in a desiccator.

**16.2** Allow the samples to cool to room temperature in the desiccator for approximately 30 min.

**16.3** Weigh the samples in the evaporating dish on the balance. Record the weight as  $W_2$  to 0.01-g accuracy.

**16.4** Repeat step **16.3** for each sample.

## 17. DATA ANALYSIS AND CALCULATIONS

**17.1** Calculate the percentage moisture content with the following formula:

$$M (\%) = \frac{W_1 - W_2}{W_1 - W_T} \times 100$$

where:

M = moisture content

$W_1$  = initial weight of smokeless tobacco sample and evaporating dish

$W_2$  = weight of dried smokeless tobacco sample and evaporating dish

$W_T$  = tare weight of evaporating dish.



The calculated moisture content can be used to convert the concentration of an analyte presented on an as-is or wet weight basis to a dry-weight basis using the following formula:

$$C_{\text{Dry}} = C_{\text{Wet}} \times \frac{100}{100 - M}$$

where:

M = moisture content (%)

$C_{\text{Dry}}$  = concentration of analyte on a dry-weight basis

$C_{\text{Wet}}$  = concentration of analyte on an as-is or wet-weight basis.

## 18. SPECIAL PRECAUTIONS

Not applicable

## 19. DATA REPORTING

- 19.1 Report individual measurements for each sample evaluated.
- 19.2 Report results as specified in the overall project specifications.
- 19.3 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

### 21.1 Limit of reporting

The limit of reporting is set to the lowest moisture content to be determined as percentage of weight.

## 21.2 Internal quality control

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:

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

**Table 1. Mean and recovery of moisture content in smokeless tobacco products**

Smokeless tobacco sample	Certified value (%)	Mean moisture content (%)	Recovery (%)
CRP 1	53.7	53.87	100.3
CRP 2	53.0	51.20	96.6
CRP 3	8.1	7.67	94.7
CRP 4	23.0	24.07	104.7

## 22. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study conducted between September 2020 and March 2021, involving 13 laboratories and 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 moisture content (%) in smokeless tobacco products**

Reference tobacco product	n	Mean	Repeatability limit (r)	Reproducibility limit (R)
CRP1.1	9	53.87	1.02	1.93
CRP2.1	10	51.20	0.76	2.73
CRP3.1	10	7.67	0.81	2.68
CRP4.1	12	24.07	1.82	4.78

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 moisture content in smokeless tobacco products.

