Cooperation Centre for Scientific Research Relative to Tobacco

Tobacco and Tobacco Products Analytes Sub-Group

CORESTA Recommended Method No. 57

DETERMINATION OF WATER IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

August 2018
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DETERMINATION OF WATER IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

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<thead>
<tr>
<th>Date of review</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 2002</td>
<td>Version 1</td>
</tr>
<tr>
<td>August 2018</td>
<td>Version 2 - Systematic review and extension of scope to include ground cigars</td>
</tr>
</tbody>
</table>
CORESTA RECOMMENDED METHOD N° 57

DETERMINATION OF WATER IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

(August 2018)

0. INTRODUCTION

This CORESTA Recommended Method specifies a gas chromatographic method for the determination of the water content of tobacco and tobacco products. This CRM is applicable to ground tobacco, a range of smokeless tobacco products, cigarette filler and ground cigar. Independent collaborative studies were conducted in 2002 and 2018. This Recommended Method has been shown to be fit for purpose for the analysis of the aforementioned matrices.

1. FIELD OF APPLICATION

This Recommended Method is applicable to raw tobacco as well as tobacco taken from finished products. The method is applicable for water contents ranging at least from a mass percent of 2 % to 55 %. The method is applicable to tobacco samples that will pass through a 4 mm screen. Repeatability and reproducibility values are included for ground tobacco, cigarette filler, cigar filler and a wide range of smokeless tobacco products. Products not covered by the collaborative studies may require additional validation.

2. DEFINITION

2.1 None

3. NORMATIVE REFERENCES


3.2 CORESTA Recommended Method N° 56: Determination of Water in Tobacco and Tobacco Products by Karl Fischer Method.


3.4 CORESTA Recommended Method N° 71: Smokeless Tobacco Products - Sampling

4. PRINCIPLE

The water content of a sample of tobacco or a tobacco product is determined by extraction in a methanol solution containing isopropanol as an internal standard, followed by gas chromatographic (GC) analysis with thermal conductivity detection (TCD). The results are reported as mass percent (%).
5. **APPARATUS**

Normal laboratory apparatus, and in particular, the following items:

5.1 Analytical balance (0.0001 g accuracy).

5.2 Extraction vessels. Dry serum bottles with crimp caps or 125 ml conical flasks with ground glass stoppers.

5.3 Volumetric pipettes, of appropriate volumes.

5.4 Mechanical shaker, adjustable to a shaking frequency at a rate that will ensure sufficient extraction.

5.5 Volumetric flasks, of capacities 100 ml, 250 ml and 500 ml.

5.6 Dispensing pipette, of capacity 100 ml, for organic solvents.

5.7 Drying tube for dispenser.

5.8 Gas chromatograph, equipped with a thermal conductivity detector, autosampler, and data collection system.

5.9 GC column: Plot column (25 m x 0.53 mm I.D., 20 µm film thickness)\(^1\); see the attached Annex 1.

5.10 Inlet liner for injection port, deactivated glass.

6. **REAGENTS**

All reagents must be of recognized analytical grade and comply with existing national regulations.

6.1 Carrier gas: helium or nitrogen.

6.2 Methanol, (analytical grade, maximum water content of 1.0 mg/ml).

6.3 Internal Standard, isopropanol, ≥ 99 % purity.

6.4 Water, complying with grade 2 of ISO 3696:1987 or better.

6.5 Desiccant, Drierite\(^2\), freshly activated.

6.6 Extraction solvent, methanol containing approximately 2.0 ml of internal standard per litre.

**Note:** To ensure the homogeneity of the water content in the solvent, continuous stirring is required.

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\(^1\) The following column is an example of a suitable product available commercially: PoraPLOT U, Porapak Q, and PoraPLOT Q are examples of suitable products commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

\(^2\) The following desiccant is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.
7. STANDARDS

7.1 Secondary Water Stock Solution: Transfer 25,000 g of water into a dry 500 ml volumetric flask. Dilute to volume with extraction solvent (6.6) and mix.

7.2 Working Standards: Transfer the specified volumes of secondary water stock solution (7.1) according to the table below into dry 100 ml volumetric flasks, containing approximately 25 ml of extraction solvent. Bring to a final volume with extraction solvent and mix.

Table 1 - Preparation of Working Calibration Standards

<table>
<thead>
<tr>
<th>Calibration Standards</th>
<th>Volume of 2° Water Stock (ml)</th>
<th>Final Conc. of Water (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0,0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2,5</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5,0</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>10,0</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>15,0</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>20,0</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>25,0</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>30,0</td>
</tr>
</tbody>
</table>

Note: Example calibration standards contain approximately 2,0 ml of internal standard per litre. Volume of secondary water stock solution is added to a final volume of 100 ml.

8. SAMPLING

8.1 Samples shall be stored in moisture resistant containers of sufficient size to just contain the sample. The containers shall be resistant to the migration of water and volatiles.

8.2 Frozen samples should be placed unopened in a refrigerator for a minimum of 24 hours to ensure water has fully equilibrated within the product. Next, the samples shall be removed from the refrigerator for a minimum of 2 hours prior to opening for analysis.

9. SPECIAL PRECAUTIONS

Water from the laboratory atmosphere can be adsorbed onto glassware. These factors can produce incorrect and variable results. To minimize this, the following precautions shall be taken:

9.1 Glassware and septa for vials shall be dried and stored under desiccation before use.

9.2 In order to protect the dry methanol extraction solution from ambient water vapor, the bulk extraction solvent container shall be fitted with a drying tube to prevent water being absorbed by the solvent.

Note: A desiccant gas drying tube filled with an indicating desiccant, such as Drierite, can be connected to the air inlet of a dispensing pipette or to the air inlet of a carboy. Change desiccant as needed.

9.3 Flush the extraction solvent dispensing system prior to use by dispensing to waste a minimum of 40 ml.
10. PROCEDURES

Care shall be taken during all operations to avoid contamination from atmospheric water vapor. All glassware used in the water determination shall be heated at (105 ± 5) °C for at least 1 h after all visible water has evaporated, cooled and stored in a desiccator over desiccant until used.

10.1 Sample Handling

Combine and mix sufficient tobacco to constitute at least 100 g for each test subsample. If size reduction is employed, the sample shall be reduced enough to pass through a 4 mm screen. The sample can be frozen with liquid nitrogen before cutting.

10.1.1 Portioned smokeless tobacco products shall be cut into 2 halves and added directly into the extraction vessel. Both tobacco and paper are to be analysed.

10.1.2 Cigarette filler shall be removed from the paper and filter prior to analysis. (Cut filler from cigarettes need not to be reduced in size).

Note: If a size reduction (grinding or cutting) is applied, it may create a decrease in the original water content. Cryogenic techniques may be used to prevent such losses for water.

10.2 Test Sample Preparation

10.2.1 Accurately weigh approximately 5.0 g to the nearest 1.0 mg of the sample (10.1) into a dry extraction vessel (5.2). Pipette 100.0 ml of extraction solvent (6.6) into the extraction vessel and immediately seal the vessel. A minimum of two test samples shall be prepared and analysed for each test sample.

10.2.2 Place the extraction vessel on the shaker and shake for three hours at a rate that will ensure sufficient extraction. Remove the extraction vessel from the shaker and allow it to sit overnight.

10.2.3 Gently swirl or mix the sample and then transfer an aliquot into an autosampler vial taking care not to transfer tobacco to the vial. Cap the vial immediately. Analyse the test aliquot immediately or store the extract in a refrigerator at or below 4 °C until analysis.

11. SAMPLE ANALYSIS

11.1 Gas Chromatography-TCD Operating Conditions

Set up and operate the gas chromatograph according to the manufacturer’s instructions. Condition the system just prior to use by injecting two aliquots of the extraction solvent as a primer. The following conditions are suitable for analysis:

11.1.1 Injection Parameters:
    Mode: constant flow
    Flow rate: 30 ml/min; at 50 °C
    Injection Mode: Splitless
    Inlet temp: 250 °C
    Injection volume: 1 µl injection
11.1.2 Oven Temperature:
Initial 60 °C; hold for 0 min
Ramp 5 °C/min to 130 °C
Ramp 10 °C/min to 170 °C, hold for 5 min
Run time: 23.0 min

11.1.3 Carrier Gas: Helium

11.1.4 TCD Parameters:
Reference Gas: Helium
Reference Flow: 45 ml/min
Temperature: 250 °C

Optimize the GC conditions for analyte separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards and samples, including the same injection volume.

Note: The laboratory should demonstrate that the chromatographic conditions provide sufficient resolution between water and internal standard.

Note: Nitrogen may also be used as an alternative carrier gas but may require optimization of the detector sensitivity.

11.2 Calibration of the Gas Chromatograph
Inject an aliquot of each of the calibration solutions (7) into the gas chromatograph. Record the peak areas (or height) of the water and the internal standard (6.3).

Create an internal standard calibration method in the instrument operating software. A calibration curve is generated by calculating a linear regression of the area ratios of water to internal standard as a function of the concentration ratios of water to internal standard. Ensure that the calibration curve is linear.

If the correlation coefficient R^2 is less than 0.990, then the calibration should be repeated. If an individual calibration point differs by 10 % or more from the expected value (estimated by linear regression), the problem should be investigated. The signal (peak area or height) obtained for all test aliquots must fall within the working range of the calibration curve.

11.3 Calibration Check
The full calibration procedure should be carried out daily, prior to analyses. In addition, inject an aliquot of an intermediate concentration standard after every 20 sample determinations. If the calculated concentration for this solution differs by more than 5 % from the original value, repeat the full calibration procedure.

Note: Due to the original water content of the solvent, the calibration curve will not pass through the origin.

Note: If the water content of the solvent exceeds 1.0 mg/ml, the batch should be rejected.

11.4 Blank Test
Due to the absorption of water by the solvent, duplicate blanks per set of samples are treated just as the test samples, including the shaking and transferring to injection vials.
12.  CALCULATION AND EXPRESSION OF RESULTS

12.1  Determination of the Water Content of Samples

12.1.1  Inject each test aliquot and calculate the area (or height) ratio of water to internal standard and obtain the test sample concentration in mg/ml by using the coefficients of the linear regression provided by the formula:

\[ C_t = \frac{y - b}{m} \times C_{ISTD} \]

where:
- \( C_t \) is the concentration of the test sample, in milligrams per millilitre
- \( y \) is the peak area (or height) ratio: area of analyte per area of internal standard
- \( b \) is the regression y-intercept
- \( m \) is the regression slope
- \( C_{ISTD} \) is the concentration of the internal standard in the sample extract

12.1.2  The water content, \( m\% \), of the tobacco sample expressed in mass percent (\%), is given by the formula:

\[ m\% = (C_t - C_b) \times V_t \times \left( \frac{1g}{1000mg} \right) \div m_0 \times 100 \]

where:
- \( C_t \) is the concentration of the test sample from 12.1.1, in milligrams per millilitre
- \( C_b \) is the concentration determined for the blank from 11.4, in milligrams per millilitre
- \( V_t \) is the volume of extraction solution used for the test sample, in millilitres
- \( m_0 \) is the mass of the test sample, in grams

12.2  Expression of results

Express the results to the nearest 0.1 %. 
12.3 Conversion of an analyte concentration to a dry-weight basis

The calculated water content may 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 equation:

\[ C_{\text{Dry}} = C_{\text{wet}} \times \frac{1}{(1 - m\%)} \]

where:
- \( m\% \) is the water content (%)
- \( C_{\text{Dry}} \) is the concentration of the analyte presented on a dry-weight basis
- \( C_{\text{wet}} \) is the concentration of the analyte presented on an as-is or wet-weight basis

Note: The dry-weight result will have the same units as the as-is or wet-weight result.

13. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study was conducted in 2002 that included sample types of leaf, cigarette cut filler, pipe tobacco, loose leaf chewing tobacco, and moist snuff. Both capillary and packed columns were used in this study. Twenty laboratories reported results and following the statistical analysis results from 17 laboratories were used to calculate the following mean repeatability and reproducibility limits.

Table 1 - Results of 2002 Interlaboratory Study

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Mean (%)</th>
<th>Repeatability</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( r )</td>
<td>( r ) (% of mean)</td>
</tr>
<tr>
<td>Dry Snuff</td>
<td>8,7</td>
<td>0,53</td>
<td>6,1</td>
</tr>
<tr>
<td>Leaf Burley</td>
<td>10,6</td>
<td>0,92</td>
<td>8,7</td>
</tr>
<tr>
<td>Pipe</td>
<td>11,5</td>
<td>0,78</td>
<td>6,8</td>
</tr>
<tr>
<td>Leaf Oriental</td>
<td>11,9</td>
<td>1,0</td>
<td>8,7</td>
</tr>
<tr>
<td>Cigarette Natural</td>
<td>11,8</td>
<td>0,98</td>
<td>8,3</td>
</tr>
<tr>
<td>Cigarette Menthol</td>
<td>11,5</td>
<td>0,84</td>
<td>7,3</td>
</tr>
<tr>
<td>Loose Leaf</td>
<td>23,1</td>
<td>1,3</td>
<td>5,6</td>
</tr>
<tr>
<td>Moist Snuff Long Cut 1</td>
<td>34,3</td>
<td>1,8</td>
<td>5,2</td>
</tr>
<tr>
<td>Moist Snuff Long Cut 2</td>
<td>49,1</td>
<td>1,5</td>
<td>3,0</td>
</tr>
<tr>
<td>Moist Snuff Long Cut</td>
<td>50,0</td>
<td>1,8</td>
<td>3,6</td>
</tr>
<tr>
<td>Moist Snuff Fine Cut</td>
<td>51,7</td>
<td>1,7</td>
<td>3,2</td>
</tr>
</tbody>
</table>
In 2018, the Tobacco and Tobacco Products Analytes Sub-Group (TTPA) conducted an interlaboratory study involving 10 laboratories that specified the use of this CRM\(^3\). This study included the analysis of CORESTA reference products (CRPs) manufactured in 2016, moist snuff, ground tobacco, cigarette filler, and cigar filler. Results were analysed in basic conformance with ISO 5725-2:1994 and ISO/TR 22971:2005. The mean values and \(r\) and \(R\) limits are presented in Table 2.

**Table 2 - Results from the 2018 Interlaboratory Study**

<table>
<thead>
<tr>
<th>Product Type</th>
<th>(N)(^1)</th>
<th>Mean (%)</th>
<th>Repeatability</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(r) (%)</td>
<td>(R) (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r (% of mean)</td>
<td>(R) (% of mean)</td>
<td></td>
</tr>
<tr>
<td>1R6F ground filler (Lot/Batch Number RT1) - Unflavoured American Blended cigarette filler</td>
<td>10</td>
<td>10,56</td>
<td>1,09</td>
<td>10,3</td>
</tr>
<tr>
<td>CRP1.1 - Swedish-style Snus</td>
<td>10</td>
<td>48,62</td>
<td>3,00</td>
<td>6,17</td>
</tr>
<tr>
<td>CRP2.1 - American-style loose moist snuff</td>
<td>10</td>
<td>48,01</td>
<td>2,50</td>
<td>5,22</td>
</tr>
<tr>
<td>CRP3.1 - American-style dry snuff powder</td>
<td>9</td>
<td>6,29</td>
<td>0,56</td>
<td>8,83</td>
</tr>
<tr>
<td>CRP4.1 - American-style chopped loose-leaf chewing tobacco</td>
<td>10</td>
<td>21,05</td>
<td>1,67</td>
<td>7,94</td>
</tr>
<tr>
<td>Cigar Filler #1-11/17- Flavoured cigar filler, ground</td>
<td>10</td>
<td>11,41</td>
<td>0,41</td>
<td>3,60</td>
</tr>
<tr>
<td>Cigar Filler #2-11/17- Unflavoured cigar filler, ground</td>
<td>10</td>
<td>11,45</td>
<td>0,52</td>
<td>4,56</td>
</tr>
<tr>
<td>Mentholated Cigarette-Flavoured American blended cigarette</td>
<td>10</td>
<td>9,93</td>
<td>0,78</td>
<td>7,89</td>
</tr>
<tr>
<td>MS-M, American-style loose moist snuff - Mint</td>
<td>10</td>
<td>48,55</td>
<td>2,54</td>
<td>5,23</td>
</tr>
<tr>
<td>MS-W, American-style loose moist snuff - Wintergreen</td>
<td>10</td>
<td>47,30</td>
<td>2,27</td>
<td>4,79</td>
</tr>
<tr>
<td>RT6 - Flavoured Cigar Filler</td>
<td>10</td>
<td>11,51</td>
<td>0,58</td>
<td>5,02</td>
</tr>
</tbody>
</table>

1. ‘\(N\)’ is the number of the laboratories used to determine the statistics after the removal of outliers.

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14. **TEST REPORT**

The test report shall give the water content of the sample as a mass percent (%) reported to the nearest 0.1 %. The test report shall also mention all operating conditions not specified in this CORESTA Recommended Method, or regarded as optional, as well as any circumstances that may have affected the result. It shall also include all details required for the identification of the sample.

15. **BIBLIOGRAPHY**

- CORESTA Recommended Method N° 56: *Determination of Water in Tobacco and Tobacco Products by Karl Fischer Method*.
ANNEX 1

(Informative, this Annex does not form an integral part of the Recommended Method.)

1.1. Alternative Gas Chromatographic Procedures and Analysis Precautions

Alternative gas chromatographic columns, both packed and capillary have been found suitable for the determination of water in tobacco. If these are used, it is necessary to ensure that the peaks due to water and the internal standard are well resolved from peaks due to other tobacco components and the solvent.

1.2. Alternative Columns

1.2.1 Packed Column

An example packed column would be a two-meter long stainless steel column between 2 mm and 4 mm in internal diameter, with a stationary phase of Porapak Q (80-100 mesh).

Suitable operating conditions are as follows:
- Carrier gas: helium
- Flow rate: 35 ml/min
- Injection temperature: 250 °C
- Injection volume: 2 µl
- Initial temperature: 90 °C
- Initial hold time: 2 min
- Temperature ramp: 20 °C/min
- Final temperature: 140 °C
- Final hold time: 1,5 min
- Total analysis time: 6,00 min
- Reference flow: 35 ml/min helium
- Makeup flow: 35 ml/min

1.2.2 Capillary column

Also demonstrated to be acceptable is the PLOT fused silica column, with PorapLOT Q stationary phase (20 µm film thickness), 30 m in length with 0.52 mm internal diameter.

Suitable operating conditions are as follows:
- Carrier gas: helium
- Liner velocity: at 50 °C; 30 cm/s
- Injection temperature: 250 °C
- Injection mode: splitless
- Injection volume: 1,0 µl
- Initial temperature: 75 °C
- Initial hold time: 2 min
- Temperature ramp: 10 °C/min
- Final temperature: 140 °C
- Temperature ramp B: 10 °C
- Detector: 225 °C
- Total analysis time: 7,00 min