

# CORESTA RECOMMENDED METHOD N° 57

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

*(December 2002)*

### 0. INTRODUCTION

During the development of this CORESTA Recommended Method inter-laboratory tests have been made on two different principles for the determination of the water content of raw tobacco and tobacco taken from finished products:

- the gas-chromatographic procedure, and
- the Karl Fischer procedure.

The studies show that no differences occur between the results obtained by the two different methods. The Karl Fischer method is described in CORESTA Recommended Method N° 56 *Determination of Water Content in Tobacco and Tobacco Products by Karl Fischer Method.*

### 1. FIELD OF APPLICATION

The 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 fraction of 2% to 55%. The method is applicable to any type of tobacco sample whose particle size has been reduced to totally pass through a 4 mm screen. Cut filler from cigarettes need not be reduced further 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 moisture losses.

### 2. DEFINITION

#### 2.1. *High moisture tobacco*

Any tobacco sample containing volatile matter over 20% as determined at 100 °C to 105 °C.

### 3. REFERENCES

*ISO 3696:1987*

*Water for analytical laboratory use – Specifications and test methods.*

*CORESTA Recommended Method N° 56*

*Determination of Water in Tobacco and Tobacco Products by Karl Fischer Method.*

#### 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).

#### 5. APPARATUS

Normal laboratory apparatus and in particular, the following items.

- 5.1. Extraction vessels. Dry serum bottles with crimp caps or 125 ml conical flasks with ground glass stoppers.
- 5.2. Shaker, preferably horizontal, but wrist-action is acceptable.
- 5.3. Disposable syringes, of capacity 10 ml, equipped with 25 mm membrane filters with 0,45 µm pore size, or equivalent.
- 5.4. Volumetric flasks, of capacities 100 ml, 250 ml and 500 ml.
- 5.5. Gas chromatograph, equipped with a thermal conductivity detector together with a recorder or an integrator. The recommended GC column is 25 m in length by 0,53 mm internal diameter, preferably made of PLOT fused silica. Stationary phase: PoraPLOT U 20 µm film thickness (see also the attached Annex). A suitable syringe for sample injection or for automatic analysis an autosampler, that is compatible with the gas chromatograph, is required.

**Note:** 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 CORESTA Recommended Method, and does not constitute an endorsement by CORESTA of these products.

#### 6. REAGENTS

Use only reagents of recognized analytical grade.

- 6.1. Carrier gas : helium or nitrogen (see note in 9.3).
- 6.2. Methanol, (analytical grade, maximum water content of 1,0 mg/ml).
- 6.3. Internal Standard, isopropanol, of at 99 % purity.
- 6.4. Water, complying with grade 2 of ISO 3696:1987 or better.
- 6.5. Extraction solvent, methanol containing approximately 2,0 ml of internal standard per litre.

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

## 7. STANDARDS

Prepare a series of at least four calibration solutions whose concentrations cover the range expected to be found in the test portion by adding weighed amounts of water (6.4) to the solvent (6.2). One of these calibration solutions must be the extraction solvent with no added water (solvent blank).

The calibration solutions shall be made up fresh each time extraction solution is prepared. Transfer to injection vials and cap immediately.

It is recommended that the calibration solutions be made up at least each week.

**Note:** Example calibration solutions: Make a stock standard solution by accurately weighing (to the nearest 1 mg) 25,000 g of water into a dry 500 ml volumetric flask. Dilute the water with extraction solvent (6.5) to the 500 ml mark. Pipette 0 ml, 5 ml, 10 ml, 20 ml, 30 ml, 40 ml, 50 ml, and 60 ml of the stock standard solution into each of eight different dry 100 ml volumetric flasks. Dilute to volume with extraction solvent (6.5). The working calibration solutions contain 0,0 mg, 2,5 mg, 5,0 mg, 10,0 mg, 15,0 mg, 20,0 mg, 25,0 mg, and 30,0 mg of water per ml extraction solvent.

## 8. SAMPLING

**8.1.** Each time a sample is collected and stored, it must be stored in an airtight container having a size just sufficient to contain the sample.

**8.2.** If samples are stored at or below 4 °C, allow the closed container to equilibrate at room temperature before opening.

## 9. PROCEDURES

### 9.1. *Sample Handling*

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

If high moisture samples cannot be analyzed immediately, they must be stored at or below 4 °C for no longer than 10 days.

### 9.2. *Sample Preparation*

Accurately weigh approximately 5,0 g to the nearest 1,0 mg of the sample (9.1) into a dry extraction vessel (5.1). A minimum of two test portions shall be prepared and analyzed for each test sample.

Pipette 100,0 ml of extraction solvent (6.5) into the extraction vessel and immediately seal the vessel. Place the extraction vessel on the shaker (5.2) and shake for three hours. Remove the extraction vessel from the shaker and allow it to sit overnight. Gently swirl or mix by mechanical means prior to removal of the analysis aliquot. Assemble a disposable syringe with a 0,45 µm filter (5.3). Transfer nominally 5 ml of the supernatant liquid into the disposable filtration assembly. Purge the filter of adsorbed water by disposing of a small amount of the extract. Filter the extract into a

2 ml injection vial and cap the vial immediately, making certain of tight seals. Store the filtered extract in a refrigerator at or below 4 °C until GC analysis.

### 9.3. *Gas Chromatography*

Set up and operate the gas chromatograph (5.5), recorder or integrator and autosampler (if one is used) according to the manufacturer's instructions. Ensure that the peaks for water, internal standard and solvent are well resolved. Condition the system just prior to use by injecting two 0,5 µl aliquots of the extraction solvent as a primer. Suitable operating conditions are as follows:

- Carrier gas: helium
- Linear velocity: at 50 °C; 30 cm/s
- Injection temperature: 250 °C
- Injection mode: splitless
- Injection volume: 0,5 µl
- Initial temperature: 60 °C
- Initial hold time: 0 min
- Temperature ramp A: 5 °C/min
- Final temperature A: 130 °C
- Temperature ramp B: 10 °C
- Final temperature B: 170 °C
- Final hold time B: 5 min
- Detector: 250 °C
- Total analysis time: 23 min

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 of 0,5 µl.

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

### 9.4. *Calibration of the gas chromatograph*

Inject an aliquot of each of the calibration solutions (7.0) into the gas chromatograph. Record the peak areas (or height) of the water and the internal standard (6.3). Carry out the determination at least twice, with one series interspersed with the test portion injections.

Calculate the ratio of the water peak to the internal standard peak ( $Y_i = A_{H_2O}/A_{IS}$ ) from the peak area (or height) data for each of the calibration solutions including the solvent blanks. Plot the graph of the concentrations of added water (X axis) in accordance with the area ratios (Y axis). Calculate a linear regression equation ( $Y = a + bx$ ) from this data, and use both the slope (b) and the intercept(a) of the linear regression.

If the correlation coefficient  $R^2$  is less than 0,99, then the calibration should be repeated. If an individual calibration point differs by 10 % or more from the expected value (estimated by linear regression), it should be omitted. The signal (peak area or height) obtained for all test portions must fall within the working range of the calibration curve.

### 9.5. Calibration check

The full calibration procedure should be carried out daily. 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.

If the water content of the solvent exceeds 1,0 mg/ml, the batch should be rejected.

### 9.6. Blank test

Due to the absorption of water by the solvent, duplicate blanks per set of samples are treated just as the test portions, including the shaking, filtering, and transferring to injection vials.

### 9.7. Determination of the water content of samples

9.7.1. Inject replicate aliquots of the test portion from the sample extracts. A minimum of two replicate determinations should be made under identical conditions.

**Note:** Where an autosampler is used, a single portion from the sample extracts is considered adequate.

9.7.2. Calculate the ratio of the water peak/internal standard peak ( $Y_t$ ) from the peak area (or height) data. Calculate the mass fraction for each test portion aliquot using the coefficients of the linear regression ( $m_t = (Y_t - a)/b$ )

9.7.3. The water content,  $m_w$ , of the tobacco sample expressed in milligrams per gram, is given by the equation

$$m_w = \frac{(m_t - m_b)}{m_0} \times V_t$$

where

- $m_t$  is the mass fraction of the test portion from 9.7.2, in milligrams per millilitre;
- $m_b$  is the mass fraction determined for the blank from 9.6, in milligrams per millilitre;
- $V_t$  is the volume of extracting solution used for the test portion, in millilitres;
- $m_0$  is the mass of the test portion, in grams.

## 10. 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 :

- 10.1. Glassware and septa for vials shall be dried and stored under desiccation before use.
- 10.2. The bulk solvent container shall be fitted with a trap to prevent water being absorbed by the solvent.
- 10.3. Flush the dispensing system prior to use by dispensing to waste a minimum of 40 ml.

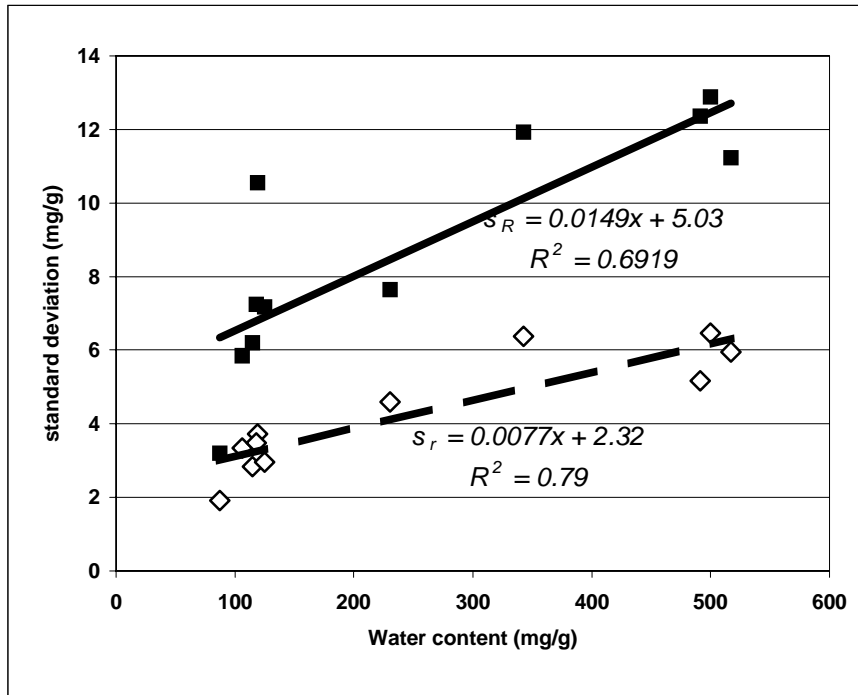
## 11. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study was conducted including 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 ( $S_r$ ) and reproducibility ( $S_R$ ), over the wide range indicated.

**Table 1 - Results of Interlaboratory tests**

sample type	H <sub>2</sub> O (mg/g)	$s_r$ (mg/g)	$s_R$ (mg/g)
dry snuff	87	1,9	3,2
leaf Burley	106	3,3	5,9
pipe	115	2,8	6,2
leaf Oriental	119	3,7	10,6
cigarette natural	118	3,5	7,3
cigarette menthol	115	3,0	7,2
loose leaf	231	4,6	7,7
moist snuff long cut 1	343	6,4	11,9
moist snuff long cut 2	491	5,2	12,4
moist snuff long cut	500	6,5	12,9
moist snuff fine cut	517	6,0	11,2

Figure 1 - Repeatability ( $s_r$ ) and reproducibility ( $s_R$ ) for H<sub>2</sub>O by gas-chromatography



## 12. TEST REPORT

The test report shall give the water content of the sample as a mass fraction in percent and the method used. 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.

## ANNEX

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

### 1. Alternative Gas Chromatographic Procedures and Analysis Precautions

#### 1.1. General

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 Columns

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