

## CORESTA RECOMMENDED METHOD N° 67

### DETERMINATION OF WATER IN THE MAINSTREAM SMOKE OF CIGARS BY GAS CHROMATOGRAPHIC ANALYSIS

*(November 2005)*

#### 1. FIELD OF APPLICATION

The method is applicable to the particulate matter of mainstream cigar smoke.

#### 2. DEFINITION

*Particulate matter* is that part of mainstream smoke which is collected by a smoke trap conforming to CORESTA Recommended Method N° 64: 2005 - Routine Analytical Cigar-Smoking Machine, Specifications, Definitions and Standard Conditions.

#### 3. REFERENCES

*CORESTA Recommended Method N° 66: 2005*

Determination of nicotine in the mainstream smoke of cigars by gas chromatographic analysis.

*CORESTA Recommended Method N° 64: 2005*

Routine analytical cigar-smoking machine: specifications, definitions and standard conditions.

*CORESTA Recommended Method N° 65: 2005*

Determination of total and nicotine-free dry particulate matter using a routine analytical cigar-smoking machine - Determination of total particulate matter and preparation for water and nicotine measurements.

*CORESTA Recommended Method N° 46: 1998*

Atmosphere for conditioning and testing cigars of all shapes and sizes.

*CORESTA Recommended Method N° 47: 2000*

Cigars – Sampling.

*CORESTA Recommended Method N° 15: 1990*

Cigarettes – Determination of water in smoke condensates – Karl Fischer method.

#### 4. PRINCIPLE

Cigars are smoked and the particulate matter of mainstream smoke is collected by a standard procedure. The particulate matter is dissolved in a solvent and the water content of this solution is determined by gas chromatography. Results are expressed as the weight of water delivered per cigar.

#### 5. APPARATUS

- 5.1. *A standard smoking machine* complying with CORESTA Recommended Method N° 64: 2005 and equipped for smoking.
- 5.2. *A gas chromatograph* equipped with a thermal conductivity detector together with a recorder and integrator or other suitable data handling instrument. Deactivated stainless steel columns for the gas chromatograph should be 1,5 m to 2 m long and have an internal diameter of about 2 mm. A suitable syringe for sample injection or for automatic analysis an autosampler, that is compatible with the gas chromatograph, is required (see Annex).
- 5.3. The necessary general laboratory equipment, for the preparation of samples, standards and reagents.

#### 6. REAGENTS

- 6.1. Propan-2-ol (analytical grade, maximum water content: 1,0 mg per ml).
- 6.2. Ethanol (minimum purity 99%).
- 6.3. Solvent for samples and standards: Propan-2-ol containing an appropriate concentration of internal standard (ethanol), normally 5 ml per litre.
- 6.4. Column packing material: Porapak Q, 80 to 100 mesh.
- 6.5. Carrier gas: helium.
- 6.6. Distilled water for the preparation of standard solutions.

#### 7. STANDARDS

Prepare a series of at least four calibration solutions whose concentrations cover the range expected to be found in the samples by adding weighed amounts of water to the solvent, described in Section 6.3. One of these calibration solutions must be the solvent with no added water (solvent blank).

## 8. PROCEDURES

### 8.1. *Gas chromatography*

Set up and operate the gas chromatograph, recorder and integrator or other suitable data handling equipment, 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.

Suitable conditions are:

Column temperature: 170 °C (isothermal)  
Injection temperature: 250 °C  
Detector temperature: 250 °C  
Carrier gas: helium at a flow rate of about 30 cm<sup>3</sup> per minute  
Injection volume: 2,0 µl

The total analysis time is about 4 minutes.

Condition the system by injecting the solvent prior to use.

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

Inject single or replicate aliquots of the standard solutions into the gas chromatograph. Record the peak areas of water and the internal standard.

Calculate the ratio of the water peak to the internal standard peak from the peak area data for each of the calibration solutions including the solvent blank. Plot the graph of the concentrations of added water in accordance with the area ratios or calculate a linear regression equation (concentration of added water in accordance with the area ratios) from these data. Use the slope of the linear regression equation.

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

### 8.3. *Calibration check*

The full calibration procedure should be carried out daily, ensuring a correlation coefficient of at least 0,99 is obtained. In addition inject an aliquot of an intermediate standard after at least every 20 samples. If the value for this solution differs by more than 3% from the original calibration value, repeat the full calibration. Check the gas chromatograph, and repeat the entire analysis.

#### 8.4. *Smoking and sample preparation*

- 8.4.1. Using CORESTA Recommended Method N° 64 and 65 (2005), set up the smoking machine, smoke cigars and collect the particulate matter.

Extract using the solvent (20 ml) described in Section 6.3. The solutions should not be stored in daylight.

The measurement can be done immediately after shaking, or after one night storing (without shaking).

- 8.4.2. Due to the absorption of water by smoke traps and solvent, it is necessary to determine a value for the sample blank. Sample blanks are prepared by treating additional smoke traps (usually four per day) in the same manner as that used for smoke collection. They are placed near the smoking machine during smoking and extracted and analysed together with the smoke samples.

The same batch of solvent shall be used for the samples as for the corresponding calibration solutions.

Prepare new calibration solutions (Section 7) whenever starting a new batch of solvent (Section 6.3).

**Note:** If measuring nicotine refer to CORESTA Recommended Method N° 66: 2005.

#### 8.5. *Measurement and calculation of the water content of samples*

- 8.5.1. Inject single or replicate aliquots of the smoke and blank solutions into the gas chromatograph using the conditions described in Section 8.1. Record the peak areas of water and the internal standard.

Calculate the mean value of the ratio of the peak area of water to that of the internal standard for the replicate injections.

- 8.5.2. Using the calibration produced in Section 8.2 determine the concentration of water in the smoke and blank solutions in mg per ml. Ensure that the values lie within the range of the standards prepared in Section 7. Determine the mean value of the sample blanks and subtract this from the values obtained for each of the smoke samples.

- 8.5.3. Calculate the water content and express the test results in milligrams per cigar, for each channel to the nearest 0,01 mg and the average per cigar to the nearest 0,1 mg.

**Note:** The minimum concentration which can be determined by this method is 0,1 mg per ml.

## 9. SPECIAL PRECAUTIONS

Water from the laboratory atmosphere can be adsorbed onto glassware and smoke traps and absorbed by solutions. These factors can produce incorrect and variable results.

To minimise 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. The bulk solvent container shall be fitted with a trap to prevent water being absorbed by the solvent.
- 9.3. Flush the dispensing system prior to use by dispensing to waste a minimum of 40 cm<sup>3</sup>.
- 9.4. All solutions shall be kept sealed.
- 9.5. The flasks used for extracting samples should not exceed 150 cm<sup>3</sup> capacity.
- 9.6. The glass fibre filter holders shall be made of a non-hygroscopic and chemically inert material, e.g. high molecular weight polyethylene.
- 9.7. The smoke traps shall be sealed until use and resealed immediately after the completion of smoking.
- 9.8. When determining nicotine and water sequentially, the water determination shall be performed first.
- 9.9. Repeat sampling should be performed with a minimum delay.
- 9.10. Suitable gloves shall be worn when handling smoke traps.

## 10. REPEATABILITY AND REPRODUCIBILITY

Collaborative Studies involving between 10 and 15 laboratories conducted in 2003/2004 show the following values for repeatability (r) and reproducibility (R) of this method.

The difference between two single results found on matched cigar samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability value (r) on average not more than once in 20 cases in the normal and correct operation of the method.

Single results on matched cigar samples reported by two laboratories will differ by more than the reproducibility value (R) on average not more than once in 20 cases in the normal and correct operation of the method.

**Table 1: Data analysis gave the estimates as summarised in the following table.**

Product	weight g	diameter mm	length mm	water mg/cigar	r	R	r relative %	R relative %
O	0,95	8,5	75,0	2,94	1,03	2,53	35	86
P	1,36	9,0	87,7	5,37	1,51	2,92	28	54
Q	1,60	9,1	99,0	6,27	1,91	5,01	30	80
K	2,11	11,1	88,0	4,99	2,31	6,70	46	134
M	3,25	14,6	95,0	8,48	2,83	5,47	33	64
R	5,10	13,7	129,5	11,16	2,92	9,21	26	83
N	6,60	15,5	127,0	11,77	3,72	9,52	32	81
S	9,60	17,0	164,0	19,49	4,59	12,86	24	66
T	11,27	17,5	162,0	49,98	17,96	38,19	36	76
C1	14,28	18,3	174,0	57,27	27,27	49,15	48	86

These findings confirm that the product variability, inherent to cigar production, is reflected in a wide range of smoke yields when cigars are machine-smoked.

For the purposes of calculating r and R, one test result (or one “single” result) was defined as the mean yield obtained from smoking 8 cigars, i.e.: 2 cigars on 4 channels, at two cigars per filter pad for Product O, P and Q. For products K, M, R, N, S, T and C1 one “single” result equally was obtained from smoking 8 cigars, but at 1 cigar per filter pad.

The subject of tolerances due to sampling is dealt with in CORESTA Recommended Method N° 47 (2000).

## 11. TEST REPORT

The test report shall give the water content from each cigar smoked and the method used, and shall include all conditions that may affect the results (e.g. atmospheric pressure during smoking). It shall also give all details necessary for the identification of the cigars smoked.

## ANNEX

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

1. The described method is a reference method that might be altered for practical purposes or special laboratory conditions in several aspects, especially:
  4. Water in smoke condensate may also be determined by the Karl Fisher method. In this case, reference to the method shall be made in the expression of results.
  - 5.2. Column tubing material other than deactivated stainless steel such as glass or nickel may be used.  
Capillary columns may be used. If these are used, it is necessary to ensure that the peaks due to water, ethanol and solvent are well separated.
  - 6.2. Methanol may be used as the internal standard.
  - 6.4. Porapak QS or Chromosorb 102 may be used as column packing material.
  - 6.5. Nitrogen may be used as an alternative carrier gas, if the detector sensitivity is sufficiently high.
2. This method can be used in conjunction with CORESTA Recommended Method N° 66: 2005 - Determination of nicotine in the mainstream smoke of cigars by gas chromatographic analysis. This may be done by:
  - (i) including the appropriate level of the internal standard required for the nicotine determination in the solvent described in Section 6.3.
  - (ii) injecting an aliquot of the smoke solution into the column for nicotine analysis that is connected to a flame ionisation detector as well as into the water column and detector described in this method. A simultaneous automated analysis of nicotine and water may be achieved by using a splitting system or an autosampler with two injection positions.