



**Cooperation Centre for Scientific Research
Relative to Tobacco**

**Tobacco and Tobacco Products Analytes
Sub-Group**

**CORESTA Recommended Method
No. 56**

**DETERMINATION OF WATER IN
TOBACCO AND TOBACCO
PRODUCTS BY KARL FISCHER
METHOD**

August 2018



CORESTA RECOMMENDED METHOD N° 56

Title:

**DETERMINATION OF WATER IN TOBACCO AND TOBACCO PRODUCTS BY
KARL FISCHER METHOD**

Status: Valid

Note:

Document history:

Date of review	Information
December 2002	Version 1
May 2011	Version 2
August 2018	Version 3 - Addition of r&R table from 2018 study including cigars

CORESTA RECOMMENDED METHOD N° 56

DETERMINATION OF WATER IN TOBACCO AND TOBACCO PRODUCTS BY KARL FISCHER METHOD

(August 2018)

0. INTRODUCTION

This CORESTA Recommended Method specifies a Karl Fischer method for the determination of the water content of tobacco and tobacco products. This CRM is applicable to ground tobaccos, a range of smokeless tobacco products, cigarette filler and ground cigars. Independent collaborative studies were conducted in 2002, 2009, 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 tobacco products and smokeless tobacco 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

- 3.1 ISO 3696:1987. *Water for analytical laboratory use – Specifications and test methods.*
- 3.2 CORESTA Guide N° 11 - *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products*
- 3.3 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 of water by shaking the sample with dry methanol, followed by injection of an aliquot portion into the titration vessel, titration with pyridine-free Karl Fischer reagent and calculation of the water content. The results are reported as mass percent (%).

5. APPARATUS

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

- 5.1. Karl Fischer apparatus for automatic titration, consisting of items 5.1.1 to 5.1.5.
 - 5.1.1 Apparatus for the detection of the titration endpoint, according to the bi-amperimetric method.
 - 5.1.2 Double electrode, made of platinum.
 - 5.1.3 Magnetic stirrer.
 - 5.1.4 Titration vessel.
 - 5.1.5 Automatic burettes, for methanol and Karl Fischer reagent.
- 5.2 Mechanical shaker, adjustable to a shaking frequency at a rate that will ensure sufficient extraction.
- 5.3 Micro-syringe, for the determination of the water equivalent, of capacity 50 µl.
- 5.4 Class A volumetric pipettes of capacities 10 ml and 20 ml.
- 5.5 Conical flasks, of capacities 250 ml and 500 ml, with a conical ground glass joint.

6. REAGENTS

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

- 6.1. Karl Fischer reagent, free from pyridine, having a water equivalent per millilitre of reagent of approximately 2 mg to 5 mg.
- 6.2. Methanol, with a water content of less than 0,05 g per 100 g.
- 6.3. Water, complying with grade 2 of ISO 3696:1987 or better.
- 6.4. Desiccant, Drierite^[1], freshly activated.
- 6.5. Gas drying unit^[2], Fisher part no. 09-204 or equivalent

7. SAMPLING

- 7.1. 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.
- 7.2. Frozen samples shall 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.

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.

^[1] 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.

^[2] The following gas drying unit is an example of a suitable product available commercially. Thermo Fisher Scientific Catalog # 09-204. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

8. PROCEDURES

Care shall be taken during all operations to avoid contamination from atmospheric moisture. 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.

Note: In order to protect the dry methanol extraction solution from ambient moisture, a desiccant gas drying jar 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.

8.1 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 if the absolute moisture level is of interest.

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

8.1.2 Cigarette filler should 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.

8.2 Test Sample Preparation

8.2.1 Weigh approximately 5 g to 0,01 g accuracy of the sample (8.1) into a dry conical flask (5.5). Add 250 ml of methanol and seal the flask immediately. Shake on the mechanical shaker (5.2) for 30 minutes with a shaking frequency at a rate that will ensure sufficient extraction

8.2.2 If a sufficiently sized sample is not available, the determination may be carried out with a reduced test portion. The minimum test portion is 0,5 g. In this case use a 250 ml conical flask and add at least 50 ml of methanol.

8.2.3 For the determination of the water content of ribs and tobacco leaves, the specified extraction time of 30 min is not sufficient. In this case extract the sample in a 500 ml conical flask with 250 ml of methanol for at least 24 hours. In some cases, a longer extraction time may be required. In this case extract the sample until constant results are obtained, i.e. the difference between the two calculations at different times is equal to or less than 0,3 g per 100 g.

8.3 Standardization of Karl Fischer reagent

Standardize the Karl Fischer reagent every working day.

8.3.1 Add sufficient methanol (6.2) to the titration vessel (5.1.4) to immerse the tips of the electrodes. Titrate any residual solution in the titration vessel to its endpoint by addition of Karl Fischer reagent (6.1).

8.3.2 Add by means of a micro-syringe (5.3), 50 µl of water to the titration vessel. To ensure that the syringe does not contain air bubbles, fill it to above the 50 µl mark, invert it and tap the air bubbles to the top. Then depress the plunger to the 50 µl mark and remove excess water quickly from the needle tip with a tissue. As an alternative, fill the syringe with 50 µl of water and weigh the syringe. After dosage, weigh the syringe again and note the exact mass of water.

8.3.3 Transfer the 50 µl of water to the titration vessel, taking care to inject the water directly into the solution, not allowing any to fall back onto the neck or walls of the vessel. Where the vessel can be fitted with a rubber membrane cap, this shall be used and the needle inserted through the cap. If a water droplet remains on the needle tip, remove it by touching the surface of the solution in the vessel.

8.3.4 Titrate with Karl Fischer reagent (\bar{V}_w) and record the titration value. Repeat the process. If the difference between the values of the two determinations is less than 0,03 ml, take the mean of the two determinations. Otherwise repeat the whole determination.

8.4 Calculation of Water Equivalent

The water equivalent E of the Karl Fischer reagent, expressed in milligrams water per millilitre, is given by the formula:

$$E = \frac{m_w}{V_w}$$

where:

m_w is the mass, in milligrams, of the volume of water used for the standardization of the Karl Fischer reagent

V_w is the mean volume, in millilitres, of the Karl Fischer reagent used for the titration of the water

Repeat the determination of the water equivalent each working day and on each new batch of Karl Fischer reagent.

8.5 Blank Test

Due to the absorption of water by the solvent, determine a value for the sample blank. Transfer 250 ml of the methanol (6.2) to a 500 ml conical flask (5.5). Using a one-mark pipette (5.4) transfer a 20 ml aliquot portion of the methanol from the conical flask to the titration vessel (5.1.4). Titrate with Karl Fischer reagent and record the value. Repeat the blank test. If the difference between the two blank tests is less than or equal to 0,05 ml, calculate the mean value. Otherwise repeat the whole determination.

The blank value, B , is given by the formula:

$$B = \frac{V_b}{V_m}$$

where

V_b is the mean volume in millilitres of the Karl Fischer reagent used for the blank test

V_m is the volume of the aliquot portion of methanol in millilitres

8.6. Determination

Measure the water content of the methanolic sample extract by injecting 10 ml into the titration vessel and titrating. After completion of the titration, discard the titrated solution to an appropriate waste container and rinse the titration vessel with methanol. A minimum of two test portions shall be prepared and analysed for each test sample. Calculate the water content.

9. CALCULATION AND EXPRESSION OF RESULTS

9.1. Determination of the water content of samples

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

$$m\% = \frac{[V_t - (B \times V_a)] \times E \times V}{m_o \times V_a} \times 100$$

where:

V_t is the volume in millilitres, of Karl Fischer reagent used for the titration of the sample extract

B is the blank value from 8.5

V_a is the volume in millilitres of the aliquot portion of the sample titrated

E is the water equivalent of the Karl Fischer reagent, in milligrams of water per millilitre of reagent

V is the total volume in millilitres, of the sample extract prepared

m_o is the mass of the test sample, in milligrams

9.2. Expression of results

Express the results to the nearest 0,1 %.

9.3. 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:

$$C_{Dry} = C_{wet} \times \frac{1}{(1 - m\%)}$$

where:

$m\%$ is the water content (%)

C_{Dry} is the concentration of the analyte presented on a dry-weight basis

C_{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.

10. 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. Eleven laboratories reported admissible results with the following estimated repeatability (r) and reproducibility (R) limits, over the wide range indicated in Table 1.

Table 1 - Results of 2002 Interlaboratory Study

Product Type	Mean (%)	Repeatability		Reproducibility	
		r	r (% of mean)	R	R (% of mean)
Dry Snuff	8,8	0,34	3,86	1,01	11,48
Leaf Burley	10,5	0,84	8,00	1,34	12,76
Pipe	11,0	0,56	5,09	1,29	11,73
Leaf Oriental	11,2	0,78	6,96	1,6	14,29
Cigarette Natural	11,6	0,76	6,55	0,95	8,19
Cigarette Menthol	12,1	0,81	6,69	0,95	7,85
Loose Leaf	22,3	0,81	3,63	1,93	8,65
Moist Snuff Long Cut 1	34,4	1,15	3,34	3,05	8,87
Moist Snuff Long Cut 2	49,4	1,4	2,83	3,25	6,58
Moist Snuff Long Cut	50,1	1,4	2,79	3,33	6,65
Moist Snuff Fine Cut	51,5	1,71	3,32	3,25	6,31

During 2009, the CORESTA Smokeless Tobacco Sub-Group (STS) conducted a Collaborative Study which included several widely-used analytical methods and nine types of smokeless tobacco^[3]. The Collaborative Study results for the determination of water in smokeless tobacco products using the Karl Fischer method are included in Table 2.

Table 2 - Results of 2009 Interlaboratory Study

Product Type	Mean (%)	N ¹	Repeatability		Reproducibility	
			r	r (% of mean)	R	R (% of mean)
Nasal Snuff	18,94	10	0,61	3,22	3,33	17,58
Loose Snus	52,76	10	1,68	3,18	6,73	12,76
Chewing Tobacco - Bits	17,76	11	1,15	6,48	2,66	14,98
Chewing Tobacco - Flake	4,15	11	0,81	19,52	2,07	49,88
Pellet	3,89	10	0,4	10,28	2,6	66,84
Chewing Tobacco - Loose Leaf	24,31	11	1,03	4,24	4,11	16,91
Moist Snuff	53,02	11	1,41	2,66	6,66	12,56
Moist Snuff	49,14	11	1,3	2,65	7,08	14,41
Pouched Snus	29,95	11	1,24	4,14	4,76	15,89

1. The number of laboratory data sets remaining after removal of outliers.

^[3] CORESTA Smokeless Tobacco Sub-Group Working Group 2: 2009 Collaborative Study Report, July 2010 (Updated January 2016).

In 2018, the Tobacco and Tobacco Products Analytes Sub-Group (TTPA) conducted an interlaboratory study involving 7 laboratories that specified the use of this CRM^[4]. 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, r and R are presented in Table 3. The value of ‘N’ is the number of the laboratories used to determine the statistics after the removal of outliers.

Table 3 - Results from the 2018 Interlaboratory Study

Product Type	N ¹	Mean (%)	Repeatability		Reproducibility	
			r	r (% of mean)	R	R (% of mean)
1R6F Ground Filler (Lot/Batch Number RT1) - Unflavoured American Blended Cigarette Filler	6	10,04	1,17	11,7	2,33	23,2
CRP1.1 - Swedish-Style Snus	6	50,03	1,43	2,9	9,85	19,7
CRP2.1 - American-Style Loose Moist Snuff	6	51,25	0,97	1,9	7,69	15,0
CRP3.1 - American-Style Dry Snuff Powder	6	6,21	0,45	7,3	2,30	37,0
CRP4.1 - American-Style Chopped Loose-Leaf Chewing Tobacco	6	21,10	0,92	4,3	1,41	6,7
Cigar Filler #1-11/17- Flavoured Cigar Filler, Ground	6	11,66	0,19	1,6	0,94	8,1
Cigar Filler #2-11/17- Unflavoured Cigar Filler, Ground	6	11,90	0,46	3,8	1,06	8,9
Mentholated Cigarette - Flavoured American Blended Cigarette	6	9,90	0,42	4,2	2,75	27,8
Rt6 - Flavoured Cigar Filler	6	11,77	0,43	3,7	0,70	6,0

1. The number of laboratory data sets remaining after removal of outliers.

11. 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.

^[4] CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: 2018 Moisture (Oven Volatiles), Water by Karl Fisher and Gas Chromatography Interlaboratory Study, August 2018.

12. BIBLIOGRAPHY

- ISO 6488:2004, *Tobacco and tobacco products – Determination of water content – Karl Fischer method.*
- CORESTA Smokeless Tobacco Sub-Group Working Group 2: 2009 Collaborative Study Report, July 2010 (Updated January 2016).
- CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: 2018 Moisture (Oven Volatiles), Water by Karl Fisher and Gas Chromatography Interlaboratory Study, August 2018.
- CORESTA Recommended Method N° 57: Determination of Water in Tobacco and Tobacco Products by Gas Chromatographic Analysis.