

CORESTA RECOMMENDED METHOD N° 56

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

(May 2011)

0. INTRODUCTION

During the development of this CORESTA Recommended Method inter-laboratory tests have been based on two different principles for the determination of the water content of raw tobacco and tobacco 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 gas chromatographic method is described in CORESTA Recommended Method N° 57, *Determination of Water in Tobacco and Tobacco Products by Gas Chromatographic Analysis*.

1. FIELD OF APPLICATION

The method is applicable to raw tobacco as well as tobacco taken from tobacco products and smokeless tobacco products. Smokeless tobacco samples supplied in the form of pouches must be analyzed with their pouch and should be cut into two halves directly into the extraction container" (but not reduced in size). 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 whose particle size has been reduced to totally pass through a 4 mm screen.

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.

Note: Smokeless tobacco products need to be sampled and handled according to CRM No. 71 and CORESTA Guide N° 11.

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

3.1 *ISO 3696:1987. Water for analytical laboratory use – Specifications and test methods.*

3.2 *CORESTA Recommended Method N° 57: Determination of Water in Tobacco and Tobacco Products by Gas Chromatographic Analysis.*

3.3 *CSTS Smokeless Tobacco Glossary*

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.

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 of 155 min⁻¹
- 5.3. Micro-syringe, for the determination of the water equivalent, of capacity 50 µl.
- 5.4. One-mark pipettes, of capacities 10 ml and 20 ml, complying with Class A of ISO 648.
- 5.5. Conical flasks, of capacities 250 ml and 500 ml, with a conical ground glass joint.

6. REAGENTS

Use only reagents of recognized analytical grade.

- 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, silica gel, freshly activated.
- 6.5. Gas drying jar, Fisher part no. 09-204 or equivalent

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.

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

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 $(105 \pm 5) ^\circ\text{C}$ for at least 1 h after all visible water has evaporated, cooled and stored in a desiccator over silica gel until used.

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 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 $4 ^\circ\text{C}$ for no longer than 10 days.

The Snus pouches should be cut into 2 halves directly into the extraction vessel. Both Snus and paper are to be analysed.

8.2. *Test Portion 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 of 155 min^{-1} .

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*

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.

Standardize the Karl Fischer reagent every working day.

8.4. *Calculation of Water Equivalent*

The water equivalent E of the Karl Fischer reagent, expressed in milligrams H_2O per millilitre, is given by the equation

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

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 daily 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 equation

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

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. Repeat the determination. Calculate the water content. If the difference between the two calculations is less than or equal to 0.3 g per 100 g, calculate the mean value. Otherwise repeat the whole determination.

9. CALCULATION AND EXPRESSION OF RESULTS

9.1. Method of Calculation

The water content W_t of the tobacco, expressed as a percentage by mass, is given by the equation

$$W_t = \frac{(V_t - (B \times V_a)) \times E \times V \times 100}{m \times V_a}$$

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 (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 is the mass in milligrams, of the test portion.

9.2. Expression of results

Express the results to the nearest 0.1 % (w/w).

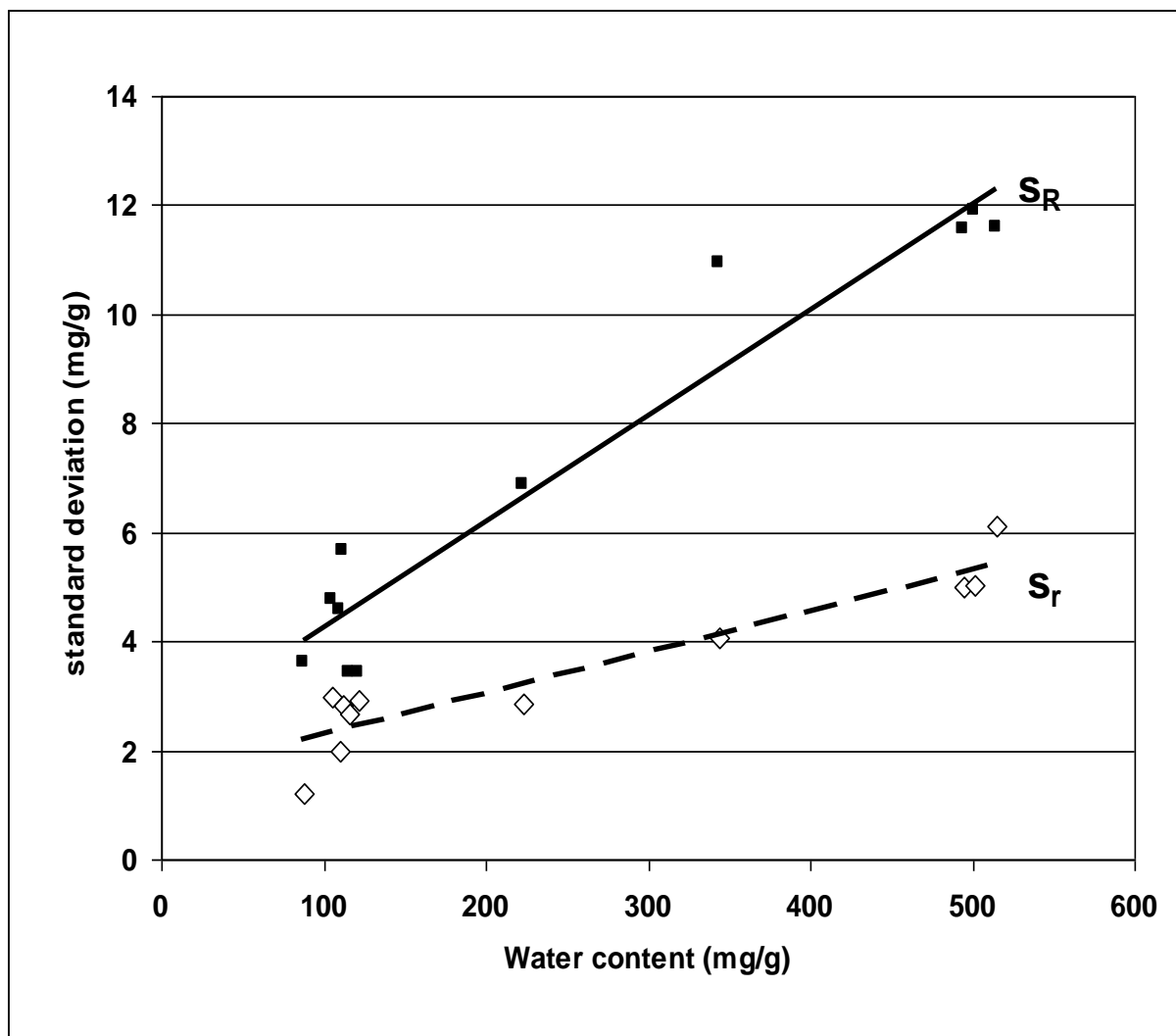
10. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study was conducted including sample types of leaf, cigarette cut filler, pipe tobacco, loose leaf chewing tobacco, and moist snuff. Eleven laboratories reported admissible results with the following mean repeatability (S_r) and reproducibility (S_R), over the wide range indicated in Table 1 and Fig.1.

Table 1 - Results of Inter-laboratory tests

Sample Type	Water (mg/g)	Repeatability S_r	Reproducibility S_R	r	R
dry snuff	88	1.2	3.6	3.4	10.1
leaf Burley	105	3.0	4.8	8.4	13.4
pipe	110	2.0	4.6	5.6	12.9
leaf Oriental	112	2.8	5.7	7.8	16.0
cigarette natural	116	2.7	3.4	7.6	9.5
cigarette menthol	121	2.9	3.4	8.1	9.5
loose leaf	223	2.9	6.9	8.1	19.3
moist snuff long cut 1	344	4.1	10.9	11.5	30.5
moist snuff long cut 2	494	5.0	11.6	14.0	32.5
moist snuff long cut	501	5.0	11.9	14.0	33.3
moist snuff fine cut	515	6.1	11.6	17.1	32.5

Figure 1 - Repeatability (S_r) and reproducibility (S_R) for H₂O by the Karl Fischer method



a $S_R = 0.0194x + 2.29$
 $R^2 = 0.930$

b $S_r = 0.0076x + 1.55$
 $R^2 = 0.868$

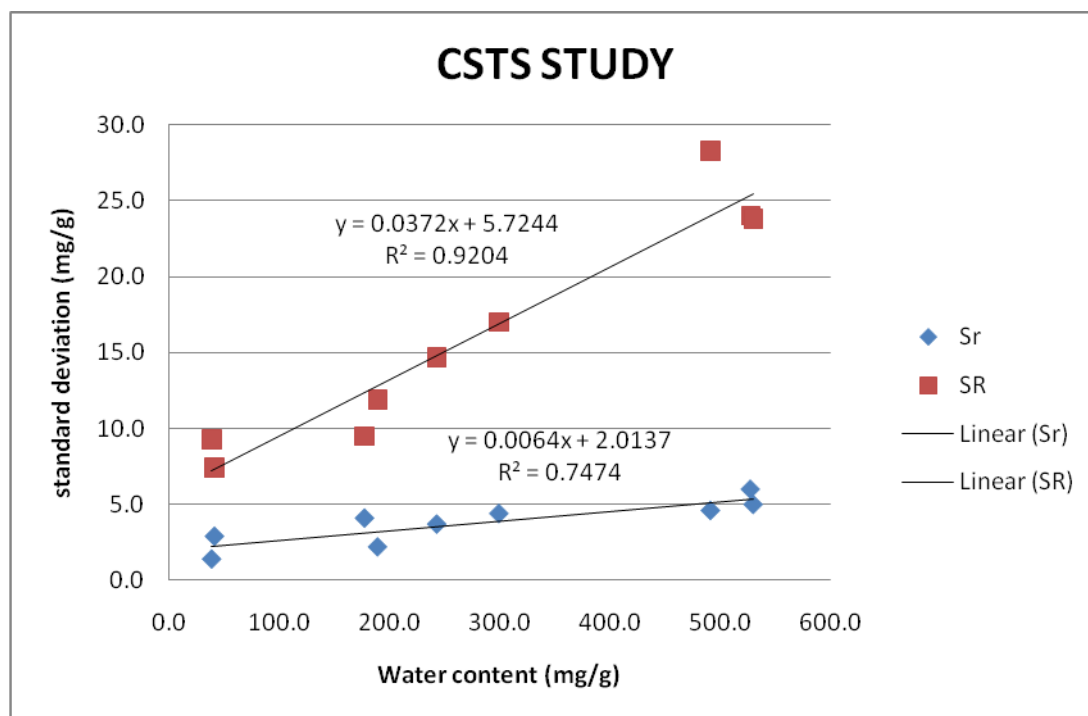
During 2009, the CORESTA Smokeless Tobacco Sub-Group (CSTS) conducted a Collaborative Study which included several widely-used analytical methods and nine types of smokeless tobacco. The Collaborative Study results for the determination of water in smokeless tobacco products using the Karl Fischer method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

Table 2 - Results of Inter-laboratory tests (2009 CSTS Collaborative Study)

Sample Type	Water mg/g	Repeatability S_r	Reproducibility S_R	r	R	N
Nasal Snuff	189.4	2.2	11.9	6.1	33.3	10
Loose Snus	527.6	6.0	24.0	16.8	67.3	10
Chewing Tobacco - Bits	177.6	4.1	9.5	11.5	26.6	11
Chewing Tobacco - Flake	41.5	2.9	7.4	8.1	20.7	11
Pellet	38.9	1.4	9.3	4.0	26.0	10
Chewing Tobacco - Loose Leaf	243.1	3.7	14.7	10.3	41.1	11
Moist Snuff	530.2	5.0	23.8	14.1	66.6	11
Moist Snuff	491.4	4.6	25.3	13.0	70.8	11
Pouched Snus	299.5	4.4	17.0	12.4	47.6	11

Supplemental information related to the description of the types of smokeless tobacco can be found in the Smokeless Tobacco Glossary (3.3).

Figure 2 - Repeatability (S_r) and reproducibility (S_R) for H_2O by the Karl Fischer method (2009 CSTS Collaborative Study)



11. 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.