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Tobacco and tobacco products — Determination of water content — Karl Fischer method

Tabac et produits du tabac — Détermination de la teneur en eau — Méthode de Karl Fischer

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

This third edition cancels and replaces the second edition (ISO 6488:2004), which has been technically revised. It also incorporates the Technical Corrigendum ISO 6488:2004/Cor 1:2008.

The main changes compared to the previous edition are as follows:

- the term high-moisture tobacco has been deleted;
- further information on interlaboratory testing including additional sample types and corresponding statistical data have been added.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document specifies a Karl Fischer method for the determination of the water content of tobacco and tobacco products. Independent collaborative studies were conducted in 2002, 2009, and 2018. This method is applicable to ground tobacco, a range of smokeless tobacco products, cigarette filler and ground cigars.

Tobacco and tobacco products — Determination of water content — Karl Fischer method

1 Scope

This document specifies a method for the determination of water content by the Karl Fischer method. It is applicable to raw tobacco as well as tobacco taken from finished products. The method is suitable for water contents ranging from a mass fraction of at least 2 % to 55 %.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 648, *Laboratory glassware — Single-volume pipettes*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

The water content of a sample of tobacco or a tobacco product is determined, using the volumetric procedure, 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 (%).

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

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Karl Fischer reagent, free from pyridine, having a water equivalent per millilitre of reagent of approximately 2 mg to 5 mg.

5.2 Methanol, with a water content of less than 0,05 g per 100 g.

Methanol is hygroscopic so it is recommended to cap the bottle with an automatic delivery pipette equipped with drying tube.

5.3 Desiccant, silica gel, freshly activated.

5.4 Water, in accordance with grade 2 of ISO 3696, or better.

6 Apparatus

Usual laboratory apparatus and, in particular, the following items.

All glassware used in the preparation and in the water determination shall be heated at (105 ± 5) °C for at least 1 h after visible water has evaporated. The glassware shall then be cooled and stored in a desiccator over desiccant (5.3) until used.

6.1 Karl Fischer apparatus for automatic titration, consisting of items 6.1.1 to 6.1.5.

6.1.1 Apparatus for the detection of the titration end point, according to the biamperiometrical method.

6.1.2 Double electrode, made of platinum.

6.1.3 Magnetic stirrer.

6.1.4 Titration vessel.

6.1.5 Automatic burettes, for methanol and Karl Fischer reagent.

6.2 Mechanical shaker, rotary platform, adjustable to a shaking frequency suitable for vigorous mixing.

6.3 Microsyringe, for the determination of the water equivalent, of capacity 50 µl.

6.4 Glass volumetric pipettes, class A, of capacities 10 ml and 20 ml, in accordance with ISO 648.

6.5 Conical flasks, of capacities 250 ml and 500 ml, with a conical ground glass joint.

6.6 Hot-air oven, capable of maintaining a temperature of (105 ± 5) °C.

7 Standardization of the Karl Fischer reagent

Add sufficient methanol (5.2) to the titration vessel (6.1.4) to immerse the tips of the electrodes. Titrate any residual solution (see NOTE) in the titration vessel to its end point by addition of Karl Fischer reagent (5.1).

Add 50 µl of water to the titration vessel using the microsyringe (6.3). 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.

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 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.

Titrate with Karl Fischer reagent (5.1) and record the titration value. Repeat the process. If the difference between the values of two determinations is not greater than 0,05 ml, take the mean of two determinations. Otherwise, repeat the whole determination.

Standardize the Karl Fischer reagent every working day.

For best results with the Karl Fischer technique, it is important to ensure that all stages of the analysis are carried out in a uniform manner, extract to extract, sample to sample, day to day.

NOTE It is common practice with the direct titration technique to carry out a titration “on top” of residual solution in the titration vessel, i.e. without removing the residual solution. When the volume in the vessel reaches a certain level, the liquid is run to waste, retaining just enough for the electrode tips to be immersed. In time, however, the methanol concentration in the vessel falls to a level such that the reaction cannot proceed satisfactorily. A precipitate can form and false titration values can be obtained. Only experience will tell when titration “on top of” residual solution has reached this point, and then the titration vessel has to be completely emptied to waste, rinsed, and recharged with methanol.

Calculate the water equivalent of the Karl Fischer reagent according to Formula (1):

$$E = \frac{m_\mathrm{w}}{V_\mathrm{w}} \qquad (1)$$

where

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

m_w is the mass, in milligrams, of the 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.

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

8 Sampling

Sampling is conducted such that the laboratory test sample is representative of the population to be tested.

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

Frozen samples should be placed unopened in a refrigerator for a minimum of 24 hours to ensure water has fully equilibrated within the product.

9 Procedure

9.1 Sample handling

Combine and mix enough retail units to constitute at least 100 g for each test subsample. If size reduction is employed, the sample should be cut sufficiently to pass through a 4 mm screen. The sample may be frozen with liquid nitrogen before cutting if the absolute moisture level is of interest. Cut filler from cigarettes need not be reduced further in size.

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

9.2 Test portion

9.2.1 It is recommended that a minimum of two test portions shall be prepared and analysed for each test sample. Take a test portion of about 5 g from the sample that has been prepared according to 9.1. Weigh to the nearest 0,001 g and transfer this test portion to a 500 ml conical flask (6.5). Add (250 ± 1) ml of methanol to the flask and close the flask immediately. Shake on the mechanical shaker (6.2) for 30 min, with a shaking frequency of 155 min^{-1}or at a rate to sufficiently to agitate the samples.

9.2.2 If a sufficiently sized sample is not available, the determination may also 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.

9.2.3 For the determination of the water content of tobacco stems and tobacco leaves, an 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, shake for 30 min and let stand for at least 24 h. The test portions should be gently swirled or mixed mechanically prior to removal of the analysis aliquot. In special cases a longer extraction time may be necessary. 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 0,5 g per 100 g.

9.2.4 If the extract is not analysed on the same day, store in a refrigerator. The sample shall be allowed to equilibrate to ambient conditions prior to analysis.

9.3 Preparation of titration apparatus

Prepare the titration apparatus in accordance with the instructions for use. Add sufficient methanol (5.2) to the titration vessel (6.1.4) so that the tips of the platinum double electrode are completely immersed during stirring. Titrate the contents of the titration vessel to the end-point by addition of Karl Fischer reagent.

9.4 Blank test

Transfer (250 ± 1) ml of the methanol (5.2) to a 500 ml conical flask (6.5), and take it through the same operations as the sample extractions (9.2). Take from the conical flask an aliquot portion of 20,0 ml of the methanol using a volumetric pipette (6.4) and transfer to the titration vessel (6.1.4). Titrate with Karl Fischer reagent and record the value. Repeat the blank test for the second replicate. If the difference between the values of two blank replicates 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 Formula (2):

$$B = \frac{V_\mathrm{b}}{V_\mathrm{m}} \tag{2}$$

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.

9.5 Determination

Transfer 10 ml of the methanolic sample extract into the titration vessel using a volumetric pipette (6.4) and titrate. After completion of the titration, remove the titrated solution and rinse the titration vessel with methanol. Repeat the determination for the additional replicate. Calculate the water content. If the difference between the two replicates is less than or equal to 0,5 g per 100 g (i.e. 0,5 %), calculate the mean value. Otherwise, repeat the whole determination.

10 Expression of results

The water content of tobacco, w_T, expressed as mass percent (%), is given by Formula (3):

$$w_T = \frac{\left[V_t - \left(B \cdot V_a\right)\right] \cdot E \cdot V}{m \cdot V_a} \times 0{,}1 \qquad (3)$$

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 (9.4);

V_a is the volume of the aliquot portion, in millilitres, of the sample extract titrated;

E is the water equivalent of the Karl Fischer reagent, in milligrams of water per millilitre of reagent (Clause 7);

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

m is the mass, in grams, of the test portion;

0,1 is the factor to convert (W_T) from milligrams per gram to mass percent (%).

Express the result to the nearest 0,1 %.

11 Repeatability and reproducibility

An international collaborative study was conducted in 2002 which included ground leaf, cigarette cut filler, pipe tobacco, loose-leaf chewing tobacco and moist snuff. A statistical analysis of results from 11 laboratories was conducted using ISO 5725-2 procedures to calculate mean values and repeatability and reproducibility as shown in Table 1.

Table 1 — Results of 2002 interlaboratory study

Product type	Mean water content (%)	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	1,40	2,83	3,25	6,58
Moist snuff long cut	50,1	1,40	2,79	3,33	6,65
Moist snuff fine cut	51,5	1,71	3,32	3,25	6,31

A second interlaboratory study was conducted in 2009. The 2009 study involved 11 laboratories and included the analysis of nine types of smokeless tobacco[3]. Results were analysed in basic conformance with ISO 5725-2 and ISO/TR 22971. The mean values, r and R are presented in Table 2. The value of "N" is the number of the laboratories used to determine the statistics after the removal of outliers.

Table 2 — Results of 2009 interlaboratory study

Product type	Mean water content (%)	N[a]	Repeatability		Reproducibility	
			r	r (% of mean)	R	R (% of mean)
Pellet	3,89	10	0,4	10,28	2,6	66,84
Chewing tobacco – Flake	4,15	11	0,81	19,52	2,07	49,88
Chewing tobacco – Bits	17,76	11	1,15	6,48	2,66	14,98
Nasal snuff	18,94	10	0,61	3,22	3,33	17,58
Chewing tobacco – Loose leaf	24,31	11	1,03	4,24	4,11	16,91
Pouched snus	29,95	11	1,24	4,14	4,76	15,89
Moist snuff – Loose long cut	49,14	11	1,3	2,65	7,08	14,41
Loose snus	52,76	10	1,68	3,18	6,73	12,76
Moist snuff – Loose	53,02	11	1,41	2,66	6,66	12,56

[a] The number of laboratory data sets after removal of outliers.

A third interlaboratory study was conducted in 2018. The 2018 study involved 6 laboratories and included the analysis of CORESTA reference products (CRPs) manufactured in 2016, moist snuff, ground tobacco, cigarette filler, and cigar filler[4]. Results were analysed in basic conformance with ISO 5725-2 and ISO/TR 22971. 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 of 2018 interlaboratory studies

Product type	Mean water content (%)	N[a]	Repeatability		Reproducibility	
			r	r (% of mean)	R	R (% of mean)
1R6F Ground Filler (RT1) – American clended cigarette filler	10,04	6	1,17	11,7	2,33	23,2
CRP1.1 – Swedish-style snus	50,03	6	1,43	2,9	9,85	19,7
CRP2.1 – American-style loose moist snuff	51,25	6	0,97	1,9	7,69	15,0
CRP3.1 – American-style dry snuff powder	6,21	6	0,45	7,3	2,30	37,0
CRP4.1 – American-style chopped loose-leaf chewing tobacco	21,10	6	0,92	4,3	1,41	6,7
Cigar filler #1 – Flavoured cigar filler, ground	11,66	6	0,19	1,6	0,94	8,1
Cigar filler #2 – Unflavoured cigar filler, ground	11,90	6	0,46	3,8	1,06	8,9
Mentholated cigarette – American blended cigarette	9,90	6	0,42	4,2	2,75	27,8
Rt6 – Flavoured cigar filler	11,77	6	0,43	3,7	0,70	6,0

[a] The number of laboratory data sets after removal of outliers.

12 Test report

The test report shall show:

a) the water content of the test sample, as mass percent (%) reported to the nearest 0,1 %, and the method used;