INTERNATIONAL STANDARD

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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

Reference number
ISO 6488:2004(E)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 6488 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

This second edition of ISO 6488 cancels and replaces ISO 6488-1:1997, which has been technically revised.

Introduction

During the development of International Standards, inter-laboratory tests were carried out using two different principles for the determination of water content of raw tobacco and tobacco taken from finished products. These were

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

These studies show that no differences occur between the results obtained by the two different methods. The gas chromatographic method is described in ISO 16632.

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

1 Scope

This International Standard 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 referenced documents are indispensable for the application 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 — One-mark pipettes*

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

ISO 4874, *Tobacco — Sampling of batches of raw material — General principles*

ISO 8243, *Cigarettes — Sampling*

ISO 10362-2, *Cigarettes — Determination of water in smoke condensates — Part 2: Karl Fischer method*

ISO 15592-1, *Fine-cut tobacco and smoking articles made from it — Methods of sampling, conditioning and analysis — Part 1: Sampling*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
high-moisture tobacco
any tobacco sample containing volatile matter over 20 % as determined by drying at between 100 °C and 105 °C

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 method is applicable to any type of tobacco sample provided that the particle size reduction is less than 4 mm.

NOTE 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.

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

WARNING — Methanol is hazardous to human subjects and to the environment. Handle it with care.

5.3 Desiccant, silica gel, freshly activated.

5.4 Water, complying 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 endpoint, 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 of 155 min^{-1}.

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

6.4 One-mark pipettes, of capacities 10 ml and 20 ml, complying with Class A of 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 endpoint 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 may form and false titration values may 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 the following equation:

$$E = \frac{m_{\mathrm{w}}}{V_{\mathrm{w}}} \tag{1}$$

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

Sample raw tobacco in accordance with ISO 4874 and cigarettes in accordance with ISO 8243. Sample fine-cut tobacco in accordance with ISO 15592-1.

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

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

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.

If high-moisture samples (see 3.1) cannot be analysed immediately, they should be stored below 4 °C for no longer than 10 days.

9.2 Test portion

9.2.1 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}.

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. After conditioning to room temperature, the extract should be analysed.

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 one-mark 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. If the difference between the values of two determinations 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

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

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 one-mark pipette (6.4) and titrate. After completion of the titration, remove the titrated solution 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 0,5 g per 100 g, calculate the mean value. Otherwise repeat the whole determination.

10 Expression of results

The water content of tobacco, w_T, expressed as milligrams per gram, is given by the equation

$$w_\mathrm{T} = \frac{[V_\mathrm{t} - (B \cdot V_\mathrm{a})] \cdot E \cdot V}{m \cdot V_\mathrm{a}} \tag{3}$$

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.

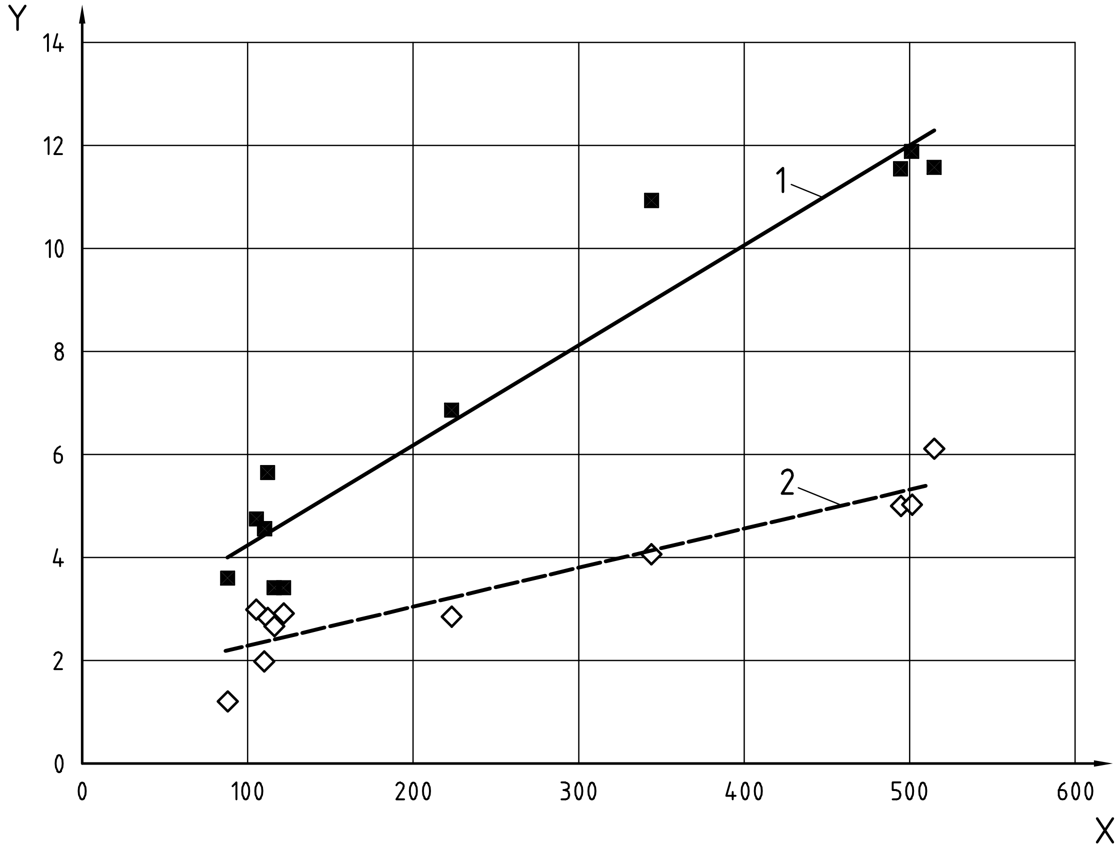
Express the result to the nearest milligram per gram.

11 Repeatability and reproducibility

An international collaborative study was conducted which 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 mean standard deviations of repeatability (s_r) and reproducibility (s_R), over the wide range indicated. The determination of repeatability and reproducibility limits of the Karl Fischer method were performed in accordance with ISO 5725-2.

Table 1 — Results of interlaboratory tests

Tobacco sample type	**Water content** mg/g	s_r mg/g	s_R mg/g
Dry snuff	88	1,2	3,6
Leaf Burley	105	3,0	4,8
Pipe	110	2,0	4,6
Leaf Oriental	112	2,8	5,7
Cigarette, natural	116	2,7	3,4
Cigarette, menthol	121	2,9	3,4
Loose leaf	223	2,9	6,9
Moist snuff, long cut 1	344	4,1	10,9
Moist snuff, long cut 2	494	5,0	11,6
Moist snuff, long cut	501	5,0	11,9
Moist snuff, fine cut	515	6,1	11,6



Key

X water content, mg/g

Y standard deviation, mg/g

1 $s_R = 0{,}019\,4x + 2{,}29$
$R^2 = 0{,}930$

2 $s_r = 0{,}007\,6x + 1{,}55$
$R^2 = 0{,}868$

Figure 1 — Standard deviations of repeatability (s_r) and reproducibility (s_R) for water content by the Karl Fischer method

12 Test report

The test report shall show the water content of the test sample, in milligrams per gram, and the method used. Reference shall be made to this International Standard. The test report shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have affected the result.

The test report shall also include all details required for the complete identification of the sample.

Bibliography

[1] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[2] ISO 16632, *Tobacco and tobacco products — Determination of water content — Gas-chromatographic method*

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