INTERNATIONAL STANDARD

ISO 6488-1

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Tobacco — Determination of water content —

Part 1:
Karl Fischer method

Tabac — Détermination de la teneur en eau —
Partie 1: Méthode de Karl Fischer

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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.

International Standard ISO 6488-1 was prepared by Technical Committee ISO/TS 126, *Tobacco and tobacco products*, Subcommittee SC 2, *Leaf tobacco*.

ISO 6488 consists of the following parts, under the general title *Tobacco — Determination of water content*:

— *Part 1: Karl Fischer method*

— *Part 2: Azeotropic entrainment method*

Part 2 (to be published) will be a revision of ISO 6488:1981.

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Tobacco — Determination of water content —

Part 1:
Karl Fischer method

1 Scope

This part of ISO 6488 specifies a method for the determination of water content by the Karl Fischer method in uncased tobacco in any form.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 6488. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 6488 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 648:1977, *Laboratory glassware — One-mark pipettes.*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 4874:1981, *Tobacco — Sampling of batches of raw material — General principles.*

3 Definitions

For the purposes of this part of ISO 6488, the following definitions apply.

3.1 water content of tobacco: Proportion of water extracted by dried methanol from the sample, as determined by the method specified in this part of ISO 6488.

3.2 uncased tobacco: Tobacco to which no flavouring material, hydroscopic agent, etc., is added.

4 Principle

Extraction of water by shaking the sample with dry methanol. Injection of an aliquot portion into the titration vessel. Titration with pyridine-free Karl Fischer reagent and calculation of the water content.

5 Reagents

During the analysis, 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.

5.3 Desiccant, such as silica gel, freshly activated.

5.4 Water, conforming to grade 2 of ISO 3696.

6 Apparatus

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, 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 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 0,03 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 sufficient 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 be obtained. Only experience will tell when titrating “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

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

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 Procedure

8.1 Sampling

Sampling shall be carried out in accordance with ISO 4874.

8.2 Test portion

8.2.1 Take a test portion of about 5 g from the sample that has been prepared according to 8.1. Weigh to the nearest 0,001 g and transfer this test portion to one of the conical flasks (6.5). Add 250 ml of methanol and close the flask immediately. Shake on the mechanical shaker (6.2) for 30 min, with a shaking frequency of 155 min^{-1}.

8.2.2 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.

8.2.3 For the determination of the water content of ribs 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 for at least 24 h. 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 0,3 g per 100 g.

8.3 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.

8.4 Blank test

Transfer 250 ml of the methanol (5.2) to a 500 ml conical flask (6.5). Take from the conical flask an aliquot portion of 20 ml of the methanol using a one-mark pipette (6.4) and transfer it to the titration vessel (6.1). Titrate with Karl Fischer reagent and record the value. Repeat the blank test. If the difference 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.

8.5 Determination

Transfer 10 ml of the methanolic sample extract into the titration vessel 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 0,3 g per 100 g, calculate the mean value. Otherwise repeat the whole determination.

9 Expression of results

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

$$w_T = \frac{\left[V_t - \left(B \times V_a\right)\right] \times E \times V \times 100}{m \times V_a}$$

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 (8.4);

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 milligrams, of the test portion.

Express the result to the nearest 0,1 %.

10 Repeatability and reproducibility

An international collaborative study was carried out to determine the repeatability (r) and reproducibility (R) limits for this method. A total of 15 laboratories took part in the collaborative study. Two batches of tobacco with different water contents were sent to participants. Each batch contained five boxes and determinations were carried out from each box under repeatability conditions. The determination of repeatability and reproducibility limits of the Karl Fischer method were performed in accordance with ISO 5725-2[1)].

Data analysis gave the estimates as summarized in table 1.

1) ISO 5725-2:1994, *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.*

Table 1

Mean value of water content (for 15 labs)	**Repeatability limit** *r*	**Reproducibility limit** *R*
13,52	0,37	0,87
16,36	0,46	1,33

The values given in table 1 shall be regarded as an upper limit as they are calculated on the basis of all 15 laboratories.

11 Test report

The test report shall state the method used and the results obtained. It shall also mention all operating conditions not specified in this part of ISO 6488, or regarded as optional, as well as any circumstances that may have affected the results. The test report shall include all details required for complete identification of the sample.

ICS 65.160

Descriptors: tobacco, tests, determination of content, water, Karl Fischer method.

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