INTERNATIONAL STANDARD

ISO 16632

Second edition
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Tobacco and tobacco products — Determination of water content — Gas-chromatographic method

Tabac et produits du tabac — Détermination de la teneur en eau — Méthode par chromatographie en phase gazeuse

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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.

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, www.iso.org/directives.

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The committee responsible for this document is ISO/TC 126, *Tobacco and tobacco products*.

This second edition cancels and replaces the first edition (ISO 16632:2003), subclause 5.2 and the bibliography of which have been technically revised and a warning notice has been added.

Introduction

During the development of this International Standard, interlaboratory tests were carried out using two different principles for the determination of the water content of raw tobacco and tobacco taken from finished products. These were

— the gas-chromatographic procedure, and

— the Karl Fischer procedure.

These studies show that no differences occur between the results obtained by the two different methods. The Karl Fischer method is described in ISO 6488.

Tobacco and tobacco products — Determination of water content — Gas-chromatographic method

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a gas-chromatographic (GC) method for the determination of water content. It is applicable to raw tobacco as well as tobacco taken from finished products. The method is suitable for water contents ranging at least from a mass fraction of 2 % to 55 %.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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 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 methanolic extraction, followed by capillary GC analysis with thermal conductivity detection, using isopropanol as internal standard. 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 Carrier gas: helium.

5.2 Methanol, with a maximum water content of 1,0 mg/ml.

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

5.3 Internal standard: isopropanol, of at least 99 % purity.

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

5.5 Extraction solvent: methanol (5.2) containing 2,0 ml of internal standard (5.3) per litre.

5.6 Desiccant: silica gel, freshly activated.

5.7 Calibration solutions

Prepare a series of at least four calibration solutions whose concentrations of added water cover the range expected to be found in the test portion by adding weighed amounts of water (5.4) to the solvent (5.5). One of these calibration solutions shall be the extraction solvent with no added water (solvent blank).

To prevent water being absorbed, the bulk solvent container shall be fitted with a water trap and all solutions shall be kept sealed. The solvent shall be stirred continuously to ensure the homogeneity of the water concentration in the solvent. The calibration solutions shall be made up using an extraction solvent from the same batch used in 8.1. Transfer them to injection vials and cap immediately.

It is recommended that the calibration solutions be made up at least each week.

NOTE For example, calibration solutions may be prepared as follows. Create a stock standard solution by accurately weighing (to the nearest 0,1 mg) 25,000 g of water into a dry 500 ml volumetric flask. Dilute the water with extraction solvent (5.5) to the 500 ml mark. Pipette 0 ml, 5 ml, 10 ml, 20 ml, 30 ml, 40 ml, 50 ml and 60 ml of the stock standard water solution into each of eight dry 100 ml volumetric flasks. Dilute to volume with extraction solvent (5.5). The standard calibration solutions contain 0,0 mg, 2,5 mg, 5,0 mg, 10,0 mg, 15,0 mg, 20,0 mg, 25,0 mg, and 30,0 mg of water per millilitre of extraction solvent.

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 (5.6) until used.

6.1 Extraction vessels, dry serum bottles with crimp caps, or conical flasks with ground glass lids, of capacity 125 ml.

It is especially important to have excellent seals to prevent water absorption from air exposure.

6.2 Shaker, preferably horizontal, but wrist-action acceptable.

6.3 Disposable syringes, of capacity 10 ml, equipped with 25 mm diameter membrane filters with 0,45 µm pore size, or equivalent.

6.4 Volumetric flasks, of capacities 100 ml, 250 ml and 500 ml.

6.5 Gas chromatograph, equipped with a thermal conductivity detector and recorder or integrator.

6.6 Column, of internal diameter 0,53 mm, and of length 25 m.

The column is preferably made of PLOT fused silica.

NOTE PoraPLOT U[1)], 20 µm film thickness, has been found to be satisfactory as the stationary phase (see also Clause 11).

7 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.

8 Procedure

8.1 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 small 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.

8.2 Test portion

Weigh, to the nearest 0,1 mg, 5,0 g of the sample (8.1) into the dry extraction vessel (6.1). A minimum of two test portions shall be prepared and analysed for each test sample.

Pipette 100,0 ml of extraction solvent (5.5) into the extraction vessel and immediately seal the vessel. Place the extraction vessel in the shaker (6.2) and shake for 3 h. Remove the extraction vessel from the shaker and set it aside overnight. The test portions should be gently swirled or mixed mechanically prior to removal of the analysis aliquot. Assemble a disposable syringe (6.3) with a 0,45 µm filter (6.3). Carefully transfer about 5 ml of the supernatant liquid into the disposable filtration assembly. Purge the filter of adsorbed water by disposing of a small volume of the extract. Filter the extract into a 2 ml GC injection vial and cap the vial. Store the filtered extract in a refrigerator below 4 °C until GC analysis, making certain of tight seals.

If the extract is not analysed on the same day, store in a refrigerator. After conditioning to room temperature, the extract should be analysed.

8.3 Setting up the apparatus

Set up the apparatus and operate the gas chromatograph (6.5), recorder or integrator and autosampler (if used) in accordance with the manufacturer's instructions. Ensure that the peaks for water, internal standard and solvent are well resolved. Condition the system just prior to use by injecting two 0,5 µl aliquots of the extraction solvent as a primer.

Suitable operating conditions are as follows:

— carrier gas: helium;

1) PoraPLOT U is an example of a suitable product commercially available. This information is given for the convenience of users of this International Standard, and does not constitute an endorsement by ISO of this product.

— linear velocity: 30 cm/s at 50 °C;

— injection temperature: 250 °C;

— injection liner: appropriate liner packed with glass wool;

— injection mode: splitless (split valve closed during injection, to be opened after about 1 min);

— injection volume: 0,5 µl;

— initial temperature: 60 °C;

— initial hold time: 0 min;

— temperature ramp A: 5 °C/min;

— final temperature A: 130 °C;

— final hold time A: 0 min;

— temperature ramp B: 10 °C/min;

— final temperature B: 170 °C;

— final hold time B: 5 min;

— total analysis time: 23,00 min;

— detector: 250 °C.

Optimize the GC conditions for analyte separation and sensitivity. Once optimized, the same GC conditions shall be used for the analysis of all standards and samples, including the same injection volume of 0,5 µl.

8.4 Calibration of the gas chromatograph

8.4.1 Procedure

Inject an aliquot (0,5 µl) of each of the calibration solutions (5.7) into the gas chromatograph. Record the peak areas (or heights) of the water and internal standard (5.3). Carry out the determination at least twice, with one series preferably interspersed with the test portion injections.

Calculate the ratio of the water peak to the internal standard peak ($Y_i = A_{H2O}/A_{IS}$) from the peak area (or height) data for each of the calibration solutions including the solvent blanks. Plot the graph of the concentrations of added water (X-axis) in accordance with the area ratios (Y-axis). Calculate a linear regression equation ($y = a + bx$) from these data, and use both the slope and the intercept of the linear regression equation.

If the correlation coefficient R^2 is less than 0,99, the calibration should be repeated. If an individual calibration point differs by 10 % or more from the expected value (estimated by linear regression), it should be omitted. The signal (peak area or height) obtained for all test portions must fall within the working range of the calibration curve.

Perform this full calibration procedure daily. In addition, inject an aliquot of an intermediate concentration standard after every 20 sample determinations. If the calculated concentration for this solution differs by more than 3 % from the original value, repeat the full calibration procedure.

Perform the full calibration if a new extraction solvent is made. If only four calibration solutions are available, no calibration solution should be omitted from the least-squares fit. The calibration curve should include the solvent blank (see 5.7).

NOTE The regression line does not pass through zero due to water present in the extraction solvent.

If the water content of the solvent exceeds 1,0 mg/ml, the batch should be rejected.

8.4.2 Blank test

Because of the absorption of water by the solvent, treat duplicate blanks per set of test samples exactly as the test portions, including shaking, filtering and transferring to injection vials.

8.4.3 Determination

Inject aliquots (0,5 µl) of the test portion (see 8.2) from the sample extracts. Calculate the ratio of the water peak/internal standard peak (Y_t) from the peak area (or height) data. Calculate the mass concentration for each test portion aliquot using the coefficients of the linear regression [$r_t = (Y_t - a)/b$].

Carry out the determination at least twice under identical conditions. Calculate the mean value of the ratio from the replicate determinations.

9 Expression of results

The water content, ω, of the tobacco sample, expressed in milligrams per gram, is given by the equation

$$\omega = \frac{(\rho_t - \rho_b)}{m_0} \cdot V_t$$

where

ρ_t is the mass concentration of the test portion from 8.4.3, in milligrams per millilitre;

ρ_b is the mass concentration determined for the blank from 8.4.2, in milligrams per millilitre;

V_t is the volume of extraction solvent used for the test portion, in millilitres;

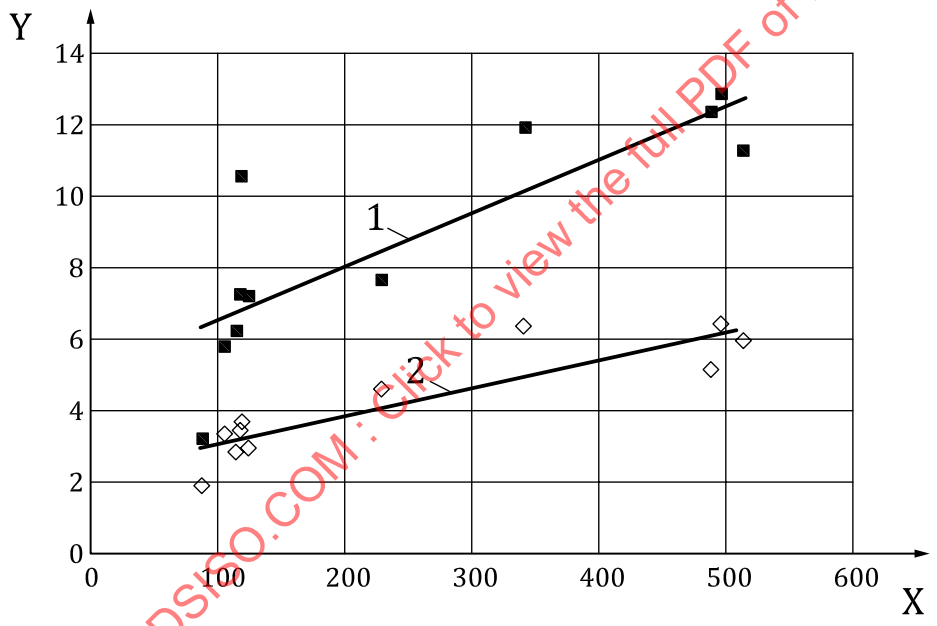
m_0 is the mass of the test portion, in grams.

10 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. Both capillary and packed columns were used in this study. Nineteen laboratories reported results with the following mean standard deviations of repeatability, s_r, and reproducibility, s_R, over the wide range indicated.

Table 1 — Results of interlaboratory tests

Tobacco sample type	Water content mg/g	s_r mg/g	s_R mg/g
Dry snuff	87	1,9	3,2
Leaf Burley	106	3,3	5,9
Pipe	115	2,8	6,2
Leaf Oriental	119	3,7	10,6
Cigarette, natural	118	3,5	7,3
Cigarette, menthol	115	3,0	7,2
Loose leaf	231	4,6	7,7
Moist snuff, long cut 1	343	6,4	11,9
Moist snuff, long cut 2	491	5,2	12,4
Moist snuff, long cut	500	6,5	12,9
Moist snuff, fine cut	517	6,0	11,2



Key

X water content, mg/g

Y standard deviation, mg/g

1 $s_R = 0{,}014\,9x + 5{,}03$
$R^2 = 0{,}691\,9$

2 $s_r = 0{,}007\,7x + 2{,}32$
$R^2 = 0{,}79$

Figure 1 — Standard deviations of repeatability, s_r, and reproducibility, s_R, for water content by gas-chromatography