



BSI Standards Publication

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

**INTERNATIONAL
STANDARD**

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16632**

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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*



Reference number
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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 (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).

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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 16632:2013), which has been technically revised.

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

- the scope of method has been expanded to include cigars and reference smokeless products;
- the reproducibility (R) and repeatability (r) tables from the 2018 international study 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 gas chromatographic method for the determination of the water content of tobacco and tobacco products. Independent collaborative studies conducted in 2002 and 2018 verified the use of this specified method for a variety of raw tobaccos and tobacco products such as smokeless tobacco, cigarette or cigar filler.

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

1 Scope

This document 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 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 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 by methanolic extraction, followed by capillary GC analysis with thermal conductivity detection, using isopropanol as internal standard.

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 or nitrogen.

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

Methanol 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 solution: methanol (5.2) containing 2,0 ml of internal standard (5.3) per litre.

The extraction solution is hygroscopic, so it is recommended to cap the bottle with an automatic delivery pipette equipped with a drying tube.

5.6 Desiccant: silica gel¹⁾, freshly activated, or other effective agents.

5.7 Calibration solutions

5.7.1 General

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 extraction solution (5.5). One of these calibration solutions shall be the extraction solution with no added water (solvent blank).

To prevent water being absorbed, the bulk extraction solution container shall be fitted with a water trap and all solutions shall be kept sealed. The extraction solution shall be stirred continuously to ensure the homogeneity of the water concentration. The calibration solutions shall be made up using an extraction solution 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.

The standard preparation procedure is given as an example and is applicable for the range of the products in a collaborative study^[1].

See [Table 1](#).

5.7.2 Water stock solution. Transfer 25,000 g of water into a dry 500 ml volumetric flask. Dilute to volume with extraction solution (5.5) and mix.

5.7.3 Working standards. Transfer the specified volumes of water stock solution (5.7.2) according to the table below into dry 100 ml volumetric flasks, containing approximately 25 ml of extraction solution (5.5). Bring to a final volume with extraction solution (5.5) and mix.

Table 1 — Preparation of working calibration standards

Calibration standards	Volume of water stock solution (ml)	Final concentration of water (mg/ml)
1	0	0,0
2	5	2,5
3	10	5,0
4	20	10,0
5	30	15,0
6	40	20,0
7	50	25,0
8	60	30,0

NOTE Example calibration standards contain approximately 2,0 ml of internal standard per litre. Volume of water stock solution is added to a final volume of 100 ml.

1) Silica gel is an example of a suitable product commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

6 Apparatus

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

All glassware used in the preparation and in the water determination shall be prepared to remove any water residue. Volumetric glassware shall be air-dried and stored in a desiccator over desiccant (5.6) until used. All other glassware 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, for example of capacity 125 ml, dry serum bottles with crimp caps, or conical flasks with ground glass lids, or equivalent.

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, equipped with membrane filters with 0,45 µm pore size, or equivalent.

6.4 Volumetric flasks, for example of capacities 100 ml and 500 ml, necessary for the preparation of the water stock solution (5.7.2) and the calibration standard solutions (5.7.3).

6.5 Gas chromatograph, equipped with a thermal conductivity detector, autosampler, and data acquisition system.

6.6 Column, a PLOT fused silica column has been demonstrated to be acceptable with PoraPLOT U²⁾ stationary phase (20 µm film thickness), 25 m in length with 0,53 mm internal diameter (see also [Clause 11](#)).

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

7 Sampling

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

8 Procedure

8.1 Sample handling

It is recommended to 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.

8.2 Sample preparation

Allow for adequate head space in the extraction vessel to increase extraction efficiency.

The sample weight and extraction volume may be adjusted on condition that it does not affect the determination.

2) PoraPLOT U with 20 µm film thickness is an example of a suitable product commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent columns may be used if they can be shown to lead to the same results, i.e. that the analytes and internal standards are sufficiently resolved from interferences.

Weigh $(5,0 \pm 0,25)$ g of the sample (8.1) into the dry extraction vessel (6.1). Record the weight to the nearest 0,000 1 g. It is recommended that a minimum of two test portions be prepared and analysed for each test sample.

The recommended procedure for portioned products such as snus is to analyse the entire portion by cutting the pouch in half and adding the tobacco and pouch material to the extraction vessel.

Pipette 100,0 ml of extraction solution (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. The sample shall be allowed to equilibrate to ambient conditions prior to analysis.

8.3 Setting up the apparatus

Set up the apparatus and operate the gas chromatograph (6.5) 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 solution as a primer.

Suitable operating conditions are as follows:

- carrier gas: helium;
- 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. An adjustment to the chromatographic conditions may be required depending on the instrument configuration and columns chosen for separation.

NOTE High boiling point components can accumulate in the column. Typically, increasing the oven temperature to 220 °C for 20 min has been found to be sufficient to avoid carry over to the next analysis.

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).

Calculate the ratio of the water peak to the internal standard peak ($Y_i = A_{H_2O}/A_{IS}$) 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. 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 solution is made. The calibration curve should include the solvent blank (i.e. extraction solution) (see 5.7).

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

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

8.4.2 Blank test

Because of the absorption of water by the extraction solution, create 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 [$r_t = (Y_t - a)/b$].

Calculate the mean value of the ratio from the replicate determinations.

Injection volume may be optimised depending upon the column specification and chromatographic conditions.

9 Expression of results

The water content, ω , of the tobacco sample, expressed as mass percent (%), is given by [Formula \(1\)](#):

$$\omega = \frac{(\rho_t - \rho_b)}{m_0} \cdot V_t \times 0,1 \quad (1)$$

where

ρ_t is the mass concentration of water in the test portion from [8.4.3](#), in milligrams per millilitre;

ρ_b is the mass concentration of water determined for the blank from [8.4.2](#), in milligrams per millilitre;

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

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

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

10 Repeatability and reproducibility

An international collaborative study was conducted in 2002 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. Twenty laboratories reported results and the statistical analysis results from 17 laboratories were used to calculate the following: mean and repeatability and reproducibility limits. See [Table 2](#).

Table 2 — Results of 2002 interlaboratory tests

Sample type	Mean water content (%)	Repeatability		Reproducibility	
		r	r	R	R
			(% of mean)		(% of mean)
Dry snuff	8,7	0,53	6,1	0,90	10,3
Leaf burley	10,6	0,92	8,7	1,7	15,6
Pipe	11,5	0,78	6,8	1,7	15,1
Leaf oriental	11,9	1,0	8,7	3,0	24,9
Cigarette natural	11,8	0,98	8,3	2,0	17,3
Cigarette menthol	11,5	0,84	7,3	2,0	17,5
Loose leaf	23,1	1,3	5,6	2,2	9,3
Moist snuff long cut 1	34,3	1,8	5,2	3,3	9,7
Moist snuff long cut 2	49,1	1,5	3,0	3,5	7,1
Moist snuff long cut	50,0	1,8	3,6	3,6	7,2
Moist snuff fine cut	51,7	1,7	3,2	3,1	6,1

In 2018, an interlaboratory study was conducted involving 10 laboratories and included the analysis of CORESTA reference products (CRPs) manufactured in 2016, moist snuff, ground tobacco, cigarette filler, and cigar filler^[1]. Results were analysed in basic conformance with ISO 5725-2^[2] and ISO/TR 22971^[3]. The mean values and r and R limits are presented in [Table 3](#).

Table 3 — Results of 2018 interlaboratory studies

Product type	N ^a	Mean water content (%)	Repeatability		Reproducibility	
			<i>r</i>	<i>r</i>	<i>R</i>	<i>R</i>
				(% of mean)		(% of mean)
1R6F ground filler (RT1) - American blended cigarette filler	10	10,56	1,09	10,3	2,67	25,3
CRP1.1 - Swedish-style Snus	10	48,62	3,00	6,17	6,56	13,5
CRP2.1 - American-style loose moist snuff	10	48,01	2,50	5,22	5,59	11,6
CRP3.1 - American-style dry snuff powder	9	6,29	0,56	8,83	1,28	20,4
CRP4.1 - American-style chopped loose-leaf chewing tobacco	10	21,05	1,67	7,94	4,87	23,1
Cigar Filler #1 - Flavoured cigar filler, ground	10	11,41	0,41	3,60	1,89	16,5
Cigar Filler #2 - Unflavoured cigar filler, ground	10	11,45	0,52	4,56	2,46	21,5
Mentholated cigarette - American blended cigarette	10	9,93	0,78	7,89	2,56	25,8
American-style loose moist snuff - Mint	10	48,55	2,54	5,23	7,87	16,2
American-style loose moist snuff - Winter-green	10	47,30	2,27	4,79	8,05	17,0
RT6 - Flavoured cigar filler	10	11,51	0,58	5,02	1,95	16,9

^a The number of laboratory data sets after removal of outliers.

11 Alternative gas-chromatographic procedures and analysis precautions

11.1 General

Alternative gas-chromatographic columns, both packed and capillary, have been found suitable for the determination of water. If alternative columns are used, it is necessary to ensure that the peaks for water and the internal standard are well resolved from peaks due to other tobacco components and the solvent.

11.2 Alternative columns

11.2.1 Packed column

An example of a suitable packed column is a 2-m long stainless-steel column, with internal diameter of between 2 mm and 4 mm, with a stationary phase of Porapak Q³⁾, 149 µm (100 mesh) to 177 µm (80 mesh).

Suitable operating conditions are as follows:

- carrier gas: helium;
- flow rate: 35 ml/min;
- injection temperature: 250 °C;
- injection volume: 2 µl;

3) Porapak Q is an example of a suitable product commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent columns may be used if they can be shown to lead to the same results, i.e. that the analytes and internal standards are sufficiently resolved from interferences.

- initial temperature: 90 °C;
- initial hold time: 2 min;
- temperature ramp: 20 °C/min;
- final temperature: 140 °C;
- final hold time: 1,5 min;
- total analysis time: 6,00 min;
- reference flow: 35 ml/min helium;
- makeup flow: 35 ml/min helium;
- detector temperature: 250 °C

11.2.2 Capillary column

The PLOT fused silica column has also been demonstrated to be acceptable with PoraPLOT Q⁴⁾ stationary phase (20 µm film thickness), 25 m in length with 0,53 mm internal diameter.

Suitable operating conditions are as follows:

- carrier gas: helium;
- linear velocity: 30 cm/s at 50 °C;
- injection temperature: 250 °C;
- injection liner: single goose neck liner packed with glass wool;
- injection mode: splitless (split valve closed time less than 1 min);
- injection volume: 1,0 µl;
- initial temperature: 75 °C;
- initial hold time: 2 min;
- temperature ramp: 10 °C/min;
- final temperature: 140 °C;
- total analysis time: 7,00 min;
- detector temperature: 250 °C.

4) PoraPLOT Q with 20 µm film thickness is an example of a suitable product commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent columns may be used if they can be shown to lead to the same results, i.e. that the analytes and internal standards are sufficiently resolved from interferences.

12 Test report

The test report shall show:

- a) the water content of the test sample, as mass % to the nearest 0,1 %, and the method used;
- b) a reference shall be made to this document, i.e. ISO 16632:2021;
- c) all operating conditions not specified in this document, or regarded as optional, as well as any circumstances that can have affected the result;
- d) all details required for the complete identification of the sample.

Bibliography

- [1] CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: 2018 Moisture (Oven Volatiles), Water by Karl Fischer and Gas Chromatography Interlaboratory Study, August 2018.
- [2] 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*
- [3] ISO/TR 22971, *Accuracy (trueness and precision) of measurement methods and results — Practical guidance for the use of ISO 5725-2:1994 in designing, implementing and statistically analysing interlaboratory repeatability and reproducibility results*
- [4] CORESTA Recommended Method No 57: Determination of Water in Tobacco and Tobacco Products by Gas Chromatographic Analysis, August 2018.

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