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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

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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).

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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 gel[1)], 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 U[2] 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 ± 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