International Standard

ISO 13110

Cigarettes — Determination of menthol in total particulate matter from mainstream cigarette smoke with a smoking regime according to ISO 3308 (standard smoking regime) — Gas chromatographic method

Cigarettes — Dosage du menthol dans la matière particulaire totale du courant principal de la fumée de cigarette avec un régime de fumage selon l'ISO 3308 (régime de fumage normalisé) — Méthode par chromatographie en phase gazeuse

Second edition
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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).

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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 second edition cancels and replaces the first edition (ISO 13110:2012), which has been technically revised.

The main changes are as follows:

— references have been included in the bibliography section for the studies in 9.1 and 9.2;

— the term “condensate” has been replaced with “total particulate matter of mainstream cigarette smoke”;

— Clause 8 has been revised to be subclause 9.1 and to include the statistical analysis methods used;

— subclause 9.2 has been added to include the r and R estimates from the CORESTA 2020 collaborative study.

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.

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Introduction

Technical Committee ISO/TC 126 coordinated a collaborative study in 2010 for the determination of menthol in mainstream cigarette smoke under ISO 4387.

The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Routine Analytical Chemistry (RAC) Sub-Group conducted a collaborative study in 2019 that used the gas chromatographic method identified in this document for the determination of menthol in mainstream cigarette smoke under ISO 3308[1] and ISO 4387. A CORESTA technical report was published in 2020 for this collaborative study involving 18 laboratories and four samples.[2]

No machine smoking regimen can represent all human smoking behaviour:

— it is recommended that cigarettes also be tested under conditions of a different intensity of machine smoking than those specified in this document;

— machine smoking testing is useful to characterize cigarette emissions for design and regulatory purposes, but communication of machine measurements to smokers can result in misunderstandings about differences in exposure and risk across brands;

— smoke emission data from machine measurements may be used as inputs for product hazard assessment, but they are not intended to be nor are they valid as measures of human exposure or risks. Communicating differences between products in machine measurements as differences in exposure or risk is a misuse of testing using ISO standards.

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Cigarettes — Determination of menthol in total particulate matter from mainstream cigarette smoke with a smoking regime according to ISO 3308 (standard smoking regime) — Gas chromatographic method

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1 Scope

This document specifies a method for the gas chromatographic determination of menthol in the total particulate matter (TPM) of mentholated cigarette mainstream smoke with a smoking regime according to ISO 3308 (standard smoking regime). The smoking of cigarettes and the collection of TPM from the mainstream smoke are carried out in accordance with ISO 4387 with some exceptions (see Annex A).

Encapsulated menthol in specific products can lead to specific handling not described in this document.

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 4387, *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine*

ISO 8243, *Cigarettes — Sampling*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology 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 TPM from the mainstream smoke is dissolved in a solvent containing an internal standard. The menthol content of an aliquot of the solution is determined by gas chromatography, and the menthol content of the TPM of the mainstream smoke is calculated and expressed as milligram per cigarette.

5 Reagents

Use only reagents of recognized analytical grade.

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5.1 Carrier gas, helium (CAS 7440-59-7), nitrogen (CAS 7727-37-9) or hydrogen (CAS 1333-74-0) of high purity.

5.2 Auxiliary gases, air and hydrogen (CAS 1333-74-0) of high purity for the flame ionization detector.

5.3 Propan-2-ol (CAS 67-63-0), methanol or ethanol may also be used.

5.4 Internal standard, *n*-heptadecane (CAS 629-78-7) of purity at least 99 %. Other appropriate internal standards (such as, but not limited to, carvone, anethole) may be used after assessment of their stability, purity and confirmation that the internal standard does not co-elute with other components of the smoke extract from the gas chromatographic column.

The peak area of internal standard in the smoke extract of samples and standards should be consistent. In cases where inconsistencies are found, analysis of an extraction of a smoke sample without the internal standard in the extraction solution should be performed to confirm the absence of a peak in the smoke extract eluting at the same time as the internal standard.

5.5 Extraction solvent, propan-2-ol (5.3) containing an appropriate mass concentration of internal standard (5.4); this is normally in the range of 0,2 mg/ml to 0,5 mg/ml. Other appropriate solvents (such as, but not limited to, ethanol, methanol) may be used after assessment of their extraction efficiency. Solvent not stored in a temperature-controlled laboratory shall be allowed to equilibrate to room temperature before use.

Room temperature should be indicative of temperatures around 22 °C. If the room temperature is substantially different from 22 °C, then all solvents and internal standards shall be tested to prove their viability for use in the method under the temperature conditions of the laboratory.

5.6 Reference substance, menthol (CAS 89-78-1), of known purity (> 99 %).

It is recommended to store menthol in an airtight container not exposed to a heat source and light. Storage at a temperature lower than 4 °C is recommended.

5.7 Calibration solutions

Dissolve the menthol (5.6) in the extraction solvent (5.5) to produce a series of at least four calibration solutions whose mass concentrations cover the range expected to be found in the test portion (usually 0,02 mg/ml to 1,0 mg/ml). Solvent and solutions stored at low temperatures shall be allowed to equilibrate to room temperature before use.

For the stock solution, store in a refrigerator and replace after a maximum of three months, or a time proven to show equivalent stability.

For the working standards, store in a refrigerator and replace after a maximum of one month or a time proven to show equivalent stability.

Room temperature should be indicative of temperatures around 22 °C. If the room temperature is substantially different from 22 °C then all solvents, internal standards, and calibration standards shall be tested to prove their viability for use in the method under the temperature conditions of the laboratory.

6 Apparatus

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

6.1 Gas chromatograph, equipped with a split/splitless multimode injector, a flame ionization detector, and a computerized controlled data acquisition and processing system.

NOTE A recorder and integrator are acceptable if proven to be operational for intended purpose.

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6.2 Column, DB-WAX[1)], 1 µm film thickness, 0,53 mm internal diameter and preferably 30 m length or any other type of column showing equivalent separation capability. An example chromatogram is given in Figure C.1.

7 Procedure

7.1 Sampling

Sampling shall be in accordance with ISO 8243.

NOTE For special precautions to be taken in sampling, see Annex B.

7.2 Test portion smoking and TPM extraction

Standard smoking of the test portion shall be in accordance with Annex A and Annex B of this document and ISO 4387. Extract by dissolving the TPM obtained by the above machine smoking of a known number of cigarettes in a fixed volume of the extraction solvent (5.5) of 20 ml for 44 mm glass fibre filter pads, or 50 ml for 92 mm glass fibre filter pads, ensuring that the glass fibre filter pad is fully covered and shake the sample on an orbital shaker or shaker shown to be equivalent for minimum of 20 min. The volume may be adjusted to give a concentration of menthol appropriate for the calibration curve (see 7.4) provided that there is adequate volume for effective extraction of the TPM of mainstream cigarette smoke.

7.3 Setting up the apparatus

Set up the apparatus and operate the gas chromatograph (6.1) in accordance with the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, menthol and other smoke component peaks are well resolved. The gas chromatograph should be equipped with an autosampler for sample injection.

Suitable operating conditions may be as follows for the column described in 6.2:

— carrier gas: helium at a flow rate of about 12 ml/min;

— make up gas: helium at a flow rate of about 5 ml/min;

— injection temperature: 200 °C;

— split ratio (approximately): 10/1;

— injection volume: 1 µl;

— oven temperature 1: 100 °C;

— time period 1 (initial): 2 min;

— temperature program 1: 10 °C/min;

— oven temperature 2: 150 °C;

— time period 2 (intermediate): 0,2 min;

— temperature program 2: 20 °C/min;

— oven temperature 3: 200 °C;

— time period 3 (final): 3 min;

— detector temperature: 250 °C.

1) DB-WAX is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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Using the above conditions, the analysis time is about 6 min to 8 min per sample. An example chromatogram is in Annex C.

NOTE The column conditions are dependent on the column specified in 6.2, or an appropriate alternative that has been tested.

7.4 Calibration of the gas chromatograph

Inject an aliquot (such as 1 µl) of each of the calibration solutions (5.7) into the gas chromatograph. Record the peak areas of the menthol and internal standard (5.4).

Calculate the ratio of the menthol peak to the internal standard peak from the peak area data for each of the calibration solutions. Generate a calibration curve by calculating a linear regression equation of the peak area ratios as a function of the concentration of menthol. The intercept of the regression line should be close to zero; if the intercept is not close to zero an investigation should occur to explain the situation, and the calibration should be repeated if necessary.

Perform this full calibration procedure daily before use. In addition, inject an aliquot of an intermediate concentration standard, which should be prepared from a separate stock, 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.

7.5 Determination

Inject aliquots (1 µl) of the TPM extract (7.2) into the gas chromatograph. Calculate the ratio of the menthol peak/internal standard peak from the peak area data and obtain the concentration of menthol in the solution by input of this ratio in the calibration curve.

8 Expression of results

From the concentration of menthol in the TPM extract, calculate the amount of menthol in the cigarette. Express the test results in milligrams per cigarette, m_M, for each channel to the nearest 0,01 mg, and the average per cigarette to the nearest 0,01 mg.

$$m_M = \frac{C\ X\ V}{n} \quad (1)$$

where

m_M is the menthol concentration in mainstream smoke TPM per cigarette;
C is the concentration of menthol in the TPM extract;
V is the volume of extraction solution, in ml (20 ml or 50 ml);
n is the number of test cigarettes smoked to generate the TPM extracted.

9 Repeatability and reproducibility

9.1 Results of the 2010 ISO/TC 126 collaborative study

An international collaborative study involving 17 laboratories and three samples, conducted in 2010, showed that when cigarettes were smoked in accordance with ISO 4387 and the resulting smoke solutions were analysed by this method, the following values for the repeatability limit (r) and the reproducibility limit (R) were obtained. The statistical evaluation was performed according to ISO 5725-2. Outlier analysis was based on the Cochran and the Grubbs' tests.

Data analysis gave the estimates as summarized in Table 1.

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