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Cigarettes — Determination of menthol in smoke condensates — Gas-chromatographic method

Cigarettes — Dosage du menthol dans les condensats de fumée — Méthode par chromatographie en phase gazeuse

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 13110 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Introduction

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 International Standard;

— 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.

Cigarettes — Determination of menthol in smoke condensates — Gas-chromatographic method

1 Scope

This International Standard specifies a method for the gas-chromatographic determination of menthol in the total particulate matter (TPM) of mentholated cigarette smoke condensates. The smoking of cigarettes and the collection of mainstream smoke are carried out in accordance with ISO 4387 with some exceptions (see Annex A).

Encapsulated menthol in specific products may lead to specific handling not described in this International Standard.

2 Normative references

The following referenced documents are indispensable for the application 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 Principle

The total particulate matter 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 total particulate matter of the mainstream smoke is calculated.

4 Reagents

Use only reagents of recognized analytical grade.

4.1 Carrier gas: helium, nitrogen or hydrogen of high purity.

4.2 Auxiliary gases: air and hydrogen of high purity for the flame ionization detector.

4.3 Propan-2-ol, methanol or ethanol may also be used.

4.4 Internal standard: *n*-heptadecane (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.

4.5 Extraction solvent: propan-2-ol (4.3) containing an appropriate mass concentration of internal standard (4.4); 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.

NOTE 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 need to be tested to prove their viability for use in the method under the temperature conditions of the laboratory.

4.6 Reference substance: menthol, of known purity (> 99 %).

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

4.7 Calibration solutions

Dissolve the menthol (4.6) in the extraction solvent (4.5) 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.

NOTE 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 need to be tested to prove their viability for use in the method under the temperature conditions of the laboratory.

5 Apparatus

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

5.1 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.

5.2 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.

6 Procedure

6.1 Sampling

Sampling is done in accordance with ISO 8243.

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

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.

6.2 Test portion

Prepare the test portion by dissolving the total particulate matter obtained by the machine smoking of a known number of cigarettes in a fixed volume of the extraction solvent (4.5) of 20 ml for 44 mm filter discs (pads), or 50 ml for 92 mm filtered discs (pads), ensuring that the disc (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 6.4) provided that there is adequate volume for effective extraction of the smoke condensate. For standard smoking, refer to ISO 4387 and Annex A of this International Standard (i.e. ISO 13110).

6.3 Setting up the apparatus

Set up the apparatus and operate the gas chromatograph (5.1) in accordance with the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, menthol and other smoke component peaks are well resolved. It is recommended to equip the gas chromatograph with an autosampler for sample injection.

Suitable operating conditions may be as follows for the column described in 5.2:

— 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.

Using the above conditions, the analysis time is about 6 min to 8 min per sample.

NOTE The column conditions are dependent on the column specified in 5.2, or an appropriate alternative that has been tested.

6.4 Calibration of the gas chromatograph

Inject an aliquot (such as 1 µl) of each of the calibration solutions (4.7) into the gas chromatograph. Record the peak areas of the menthol and internal standard (4.4).

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.

6.5 Determination

Inject aliquots (1 µl) of the test portion (6.2) 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.

7 Expression of results

From the concentration of menthol in the test portion, determine the amount of menthol in the total particulate matter. 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.

8 Repeatability and reproducibility

An international collaborative study involving 17 laboratories and three samples, conducted in 2010, showed that when cigarettes are smoked in accordance with ISO 4387 and the resulting smoke solutions are analysed by this method, the following values for the repeatability limit (r) and the reproducibility limit (R) are obtained.

Data analysis gave the estimates as summarized in Table 1.

Table 1 — Estimates given by data analysis

Brand	Mean value	Repeatability limit	Reproducibility limit
	m_M	r	R
	mg per cigarette	mg per cigarette	mg per cigarette
A	0,173	0,014	0,060
B	0,079	0,018	0,045
C	0,741	0,047	0,160

9 Test report

The test report shall state the yield of menthol per cigarette smoked and the method used, and shall include all conditions which may affect the result (e.g. test conditions, smoking regime). It shall also give all details necessary for the identification of the cigarette sample smoked.

Annex A
(normative)

Smoking procedure for the determination of menthol in smoke condensates

The cigarettes are smoked according to the procedure described in ISO 4387. However, in the determination of the menthol content, **the following exceptions** concerning the preparation and conditioning of the cigarette samples apply.

— After sample receipt, samples shall be stored in such a way as to avoid loss of menthol, and the possible contamination of non-mentholated products.

— Conditioning of the cigarettes cannot be carried out as usual due to the sublimation of menthol, therefore cigarettes have to be conditioned in their sealed pack.

— During sample preparation for smoking, samples shall be prepared as quickly as possible on the day of analysis in order to minimize loss of menthol. After sample preparation, including butt marking, is complete, the cigarettes shall be kept in a closed container until they are smoked. The maximum storage time in these containers shall not exceed 4 h.

— After the smoking process is complete, leave the cigarette butt in place for at least 30 s to enable deposition of any residual smoke in the trap.