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Cigarettes — Determination of nicotine in total particulate matter from the mainstream smoke — Gas-chromatographic method

Cigarettes — Dosage de la nicotine dans la matière particulaire totale du courant principal de fumée — Méthode par chromatographie en phase gazeuse

Reference number
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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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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 fourth edition cancels and replaces the third edition (ISO 10315:2013), which has been technically revised.

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

— The term "smoke condensate(s)" has been replaced with either “total particulate matter” or "total particulate matter from the mainstream smoke" throughout the document.

— Extraction solution and calibration solutions stored at low temperature, are equilibrated to ambient temperature before use (5.7).

— The linear regression equation for calibration is calculated by regression analysis with the area ratios in accordance with the nicotine concentrations (7.3).

— Data in Clause 9 have been replaced with the results of ISO/TR 19478-1.

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 may be considered as part of a set produced by ISO/TC 126 which describes the determination of total and nicotine-free dry particulate matter (NFDPM) in total particulate matter from the mainstream smoke. The set comprises ISO 3308, ISO 3402, ISO 4387, ISO 8243, ISO 10315 (this document) and ISO 10362-1.

A related International Standard, ISO 3400, determines total alkaloids, whereas this document determines only nicotine by virtue of the gas-chromatographic separation. Occasionally, differences can occur because of minor amounts of alkaloids other than nicotine in some types of tobacco.

Annex A provides information about the use of this method in conjunction with or simultaneously with the gas-chromatographic method of water determination specified in ISO 10362-1.

No machine smoking regime 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.

Cigarettes — Determination of nicotine in total particulate matter from the mainstream smoke — Gas-chromatographic method

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

1 Scope

This document specifies a method for the gas-chromatographic determination of nicotine in total particulate matter from the mainstream smoke. The smoking of cigarettes and the collection of mainstream smoke are carried out according to ISO 4387.

NOTE ISO 20778 and ISO 22253 provide the determination method of nicotine in smoke with an intense smoking regime.

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*

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 total particulate matter from the mainstream smoke, which shall be obtained in accordance with ISO 4387, is dissolved in an extraction solution containing an internal standard. The nicotine content of an aliquot of the smoke extract is determined by gas chromatography, and the nicotine content in the total particulate matter from the mainstream smoke is calculated.

NOTE In countries not in a position to use the gas-chromatographic method, reference is made to ISO 3400 for the determination of total nicotine alkaloids. In such cases, values obtained using the method described in ISO 3400 can be used with the addition of a note in the expression of results.

5 Reagents

Use only reagents of recognized analytical reagent grade.

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

5.3 Propan-2-ol (CAS: 67-63-0), with maximum water content of 1,0 mg/ml.

5.4 Internal standard, *n*-heptadecane (CAS: 629-78-7) or quinaldine (CAS: 91-63-4) of purity not less than 99 %.

Carvone (CAS: 99-49-0), *n*-octadecane (CAS: 593-45-3), or other appropriate internal standards may be used after assessment of their purity and determination that the internal standard does not co-elute with other components in the smoke extract. The peak area of the internal standard in smoke extracts should be monitored for consistency. 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 (see Clause 10).

5.5 Extraction solution, propan-2-ol (5.3) containing an appropriate concentration of internal standard (5.4); this is normally in the range of 0,2 mg/ml to 0,5 mg/ml.

Solution not stored in a temperature-controlled laboratory shall be allowed to equilibrate to ambient temperature before use.

5.6 Reference substance, nicotine (CAS: 54-11-5) of known purity not less than 98 %.

Store this between 0 °C and 4 °C and exclude light.

Nicotine salicylate (CAS: 29790-52-1) of known purity not less than 98 % may also be used.

The purity of the nicotine or nicotine salicylate may be verified in accordance with ISO 13276 or by any other validated method.

5.7 Calibration solutions

Dissolve the nicotine (5.6) in the extraction solution (5.5) to produce a series of at least four calibration solutions with concentrations that cover the range expected to be found in the test portion (usually 0,02 mg/ml to 2,0 mg/ml). Store these solutions at between 0 °C and 4 °C and exclude light.

Solutions stored at low temperatures shall be allowed to equilibrate to ambient temperature before use.

6 Apparatus

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

6.1 Gas-chromatograph, equipped with a flame ionization detector and a suitable data handling instrument (see Clause 10).

6.2 Column, of internal diameter between 2 mm and 4 mm and preferably of length 1,5 m to 2 m.

The column is preferably made of glass, but other materials such as deactivated stainless steel or nickel may be used. Stationary phase: 10 % poly(ethylene glycol) (PEG) 20 000 plus 2 % potassium hydroxide on an acid-washed silanized support material, 150 µm (100 mesh) to 190 µm (80 mesh) (see also Clause 10).

7 Procedure

7.1 Test portion

Prepare the test portion by dissolving the total particulate matter from the mainstream smoke obtained by the machine smoking of a known number of cigarettes in a fixed volume of the extraction solution (5.5) of 20 ml for 44 mm discs, or 50 ml for 92 mm discs, ensuring that the disc is fully covered. The volume may be adjusted to give a concentration of nicotine appropriate for the calibration graph (see 7.3) provided that there is adequate volume for effective extraction of the total particulate matter. Analysis should be performed as soon as possible, but if storage is inevitable then store the test portion at between 0 °C and 4 °C and exclude light. For standard smoking, refer to ISO 4387.

7.2 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 propan-2-ol (5.3), internal standard, nicotine, and other smoke component peaks, especially neophytadiene (which can appear on the tail of the nicotine peak under certain circumstances), are well resolved (see also Clause 10).

Suitable operating conditions are as follows:

— column temperature, 170 °C (isothermal);

— injection temperature, 250 °C;

— detector temperature, 250 °C;

— carrier gas, helium or nitrogen or hydrogen, at a flow rate of about 30 ml/min;

— injection volume, 2 µl.

Using the above conditions, the analysis time is about 6 min to 8 min (see also Clause 10).

7.3 Calibration of the gas chromatograph

Inject an aliquot (2 µl) of each of the calibration solutions (5.7) into the gas chromatograph. Record the peak areas (or heights) of the nicotine and internal standard (5.4). Carry out the determination at least twice.

Calculate the ratio of the nicotine peak to the internal standard peak from the peak area (or height) data for each of the calibration solutions. Plot the graph of the area ratios in accordance with the nicotine concentrations, and calculate a linear regression equation (area ratios according to the nicotine concentrations) from these data. The graph shall be linear and the regression line should pass through the origin. Use the reciprocal of the slope of the regression equation.

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

7.4 Determination

Inject aliquots (2 µl) of the test portion (see 7.1) into the gas chromatograph. Calculate the ratio of the nicotine peak/internal standard peak from the peak area (or height) data.

Carry out two determinations on the same test portion (see 7.1).

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

Where results are obtained from a number of separate channels of smoking and where an auto-sampler is used, a single aliquot portion from each smoke trap is considered adequate.

8 Expression of results

Calculate the concentration of nicotine in the test portion using the graph or linear regression equation prepared in 7.3. From the concentration of nicotine in the test portion, calculate the amount of nicotine in the total particulate matter. Deduce the amount in the cigarettes smoked (N). Express the test results in milligrams per cigarette, m_N, for each single result, N, to the nearest 0,01 mg and the average per cigarette to the nearest 0,1 mg.

9 Repeatability and reproducibility

A major international collaborative study involving 35 laboratories and 10 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 limits (r) and the reproducibility limits (R) are obtained. Detailed information on the scope of the study, TPM, sample descriptions and identifications is available in ISO/TR 19478-1.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval exceeds the repeatability limit (r) on average not more than once in 20 cases in the normal and correct operation of the method.

Single results on matched cigarette samples reported by two laboratories differ by more than the reproducibility limit (R) on average not more than one in 20 cases in the normal and correct operation of the method.

Data analysis gave the estimates as summarized in Table 1.

Table 1 — Estimates given by data analysis

Mean value m_N mg per cigarette	**Repeatability limit** r mg per cigarette	**Reproducibility limit** R mg per cigarette
0,108	0,025	0,046
0,154	0,016	0,057
0,389	0,040	0,087
0,663	0,076	0,120
0,665	0,051	0,123
0,677	0,054	0,126
0,752	0,058	0,126
0,816	0,072	0,130
0,830	0,055	0,126
1,366	0,088	0,164

NOTE The mean values in Table 1 are expressed in 3 digits after decimal since they are the averages of the single results (averages per cigarette) obtained at the participant laboratories for the international collaborative study.

For the purpose of calculating r and R, one test result was defined as the mean yield obtained from smoking 20 cigarettes in a single run.

For further details of the interaction of r and R with other factors, see ISO/TR 19478-1.

The subject of tolerances due to sampling is dealt with in ISO 8243.

