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Cigarettes — Determination of nicotine in total particulate matter from the mainstream smoke with an intense smoking regime — Gas-chromatographic method

Cigarettes — Dosage de la nicotine dans la matière particulaire totale provenant du courant principal de fumée avec un régime de fumage intense — Méthode par chromatographie en phase gazeuse

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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

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

Historically, a set of ISO standards have been developed to specify the requirements of analytical cigarette smoking machines and their use for the quantitative determination of a number of cigarette smoke constituents (such as total particulate matter, nicotine free dry particulate matter, water, nicotine or benzo[a]pyrene) with a unique standard smoking regime. The description of this smoking regime is provided in ISO 3308.

Later, requirements to provide smoke constituents data with an intense smoking regime, different from the ISO 3308 standard smoking regime, originated from different countries and the Conferences of the Parties to the Framework Convention on Tobacco Control, resulting in a need to specify the conditions for the use of the intense smoking regime on analytical cigarette-smoking machines. The specifications for the use of the intense smoking regime on analytical cigarette-smoking machines are provided in ISO 20778.

Elaboration of this document took into account practical work conducted in the framework of a collaborative study involving 35 laboratories (published as ISO/TR 19478-1 and ISO/TR 19478-2). It provides specifications for the gas-chromatographic determination of nicotine in total particulate matter from the mainstream smoke with an intense smoking regime.

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 with an intense smoking regime — 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 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 20779, *Cigarettes — Generation and collection of total particulate matter using a routine analytical smoking machine with an intense smoking regime*

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 http://www.electropedia.org/

4 Principle

The total particulate matter from the mainstream smoke obtained in accordance with ISO 20779 is dissolved in a solvent 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.

5 Reagents

Use only reagents of recognized analytical reagent grade.

5.1 Carrier gas, helium (CAS: 7440-59-7) or 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 solvent, 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.

Solvent not stored in a temperature-controlled laboratory shall be allowed to equilibrate to (22 ± 2) °C 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 solvent (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.

Solvent and solutions stored at low temperatures shall be allowed to equilibrate to a temperature of room in which gas-chromatography is located 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 by the intense smoking (see ISO 20779) of a known number of cigarettes in a fixed volume of the solvent (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.

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 solvent, 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, 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 traps 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. 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 with the smoking parameter mentioned in ISO 20779 (55 ml puff volume, 1 puff every 30 s, 100 % ventilation blocking) 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.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval will exceed 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 will 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
0,994	0,084	0,249
1,267	0,079	0,199
1,342	0,092	0,218
1,407	0,139	0,359
1,681	0,116	0,301
1,789	0,116	0,348
2,069	0,120	0,366
2,086	0,151	0,338
2,106	0,151	0,401
2,683	0,160	0,490

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 from a rotary machine was the mean of two runs smoking 10 test articles each and from a linear machine it was the mean of seven ports/channels, smoking three test articles per port/channel.

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

10 Alternative gas-chromatographic procedures and analysis precautions

10.1 General

Alternative gas-chromatographic columns, both packed and capillary, have been found suitable for the determination of nicotine in total particulate matter. If these are used, it is necessary to ensure that the peaks due to nicotine and the internal standard are well resolved from peaks due to other smoke components and the solvent.

10.2 Alternative columns

10.2.1 Packed columns

The following may be used as alternative stationary phases in the column described in 6.2:

— 2 % Versamid 900[1)] plus 1 % potassium hydroxide, or

— 7 % PEG 20 000 plus 3 % polyphenyl ether (6 rings), or

— lower loadings of PEG 20 000 (with or without potassium hydroxide).

10.2.2 Capillary columns

Fused silica capillary columns (0,2 mm to 0,53 mm ID) with a thin film thickness equal to or less than 1 µm, capable of analysing polar compounds, may be used.

Base-deactivated PEG stationary phases, such as CAM (J and W Scientific)[1)], Carbowax-amine (Supelco)[1)], Stabilwax-DB (Restek)[1)], and CP WAX-51 (Chrompack)[1)], give similar data to the PEG 20 000 plus potassium hydroxide packed column in 10.2.1.

10.3 Injection systems

The alternative columns described in 10.2.1 and 10.2.2 require the use of purpose-made injection systems. Suitable operating conditions may vary depending on the type of column used and they may need to be optimized following the manufacturer's instructions. Isothermal oven temperature or oven temperature programming, hold times, carrier gas and linear velocity, injection volume and split ratio shall be set for the type of capillary column used. For example, for a 15 m, 0,32 mm ID, 0,25 µm film thickness capillary column, typical conditions might be as follows:

— oven temperature: 160 °C (hold 4,5 min) rising to 200 °C at 30 °C/min (hold 1,5 min);

— carrier gas: helium at a linear flow rate of about 25 cm/s;

— split ratio: 20:1.

Using the above conditions, the analysis time is about 7 min to 8 min.

10.4 Alternative internal standards

Alternative internal standards have also been evaluated. These are carvone, quinaldine, and *n*-octadecane. These may be used after assessment of their purity and a check to ensure that they do not co-elute with other smoke components in the smoke extract being analysed. The peak area of the internal standard in smoke extracts should be monitored for consistency.

Where inconsistencies are found, analysis of a smoke sample without an 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.

11 Test report

The test report shall state the yield of nicotine per cigarette smoked and the method used and shall include all conditions which might affect the result (e.g. atmospheric test conditions during smoking). It shall also give all details necessary for the identification of the cigarettes smoked.

1) These are trade names of examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.