INTERNATIONAL STANDARD

ISO

10315

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

Cigarettes — Dosage de la nicotine dans les condensats de fumée — Méthode par chromatographie en phase gazeuse

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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.

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 10315 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

This third edition cancels and replaces the second edition (ISO 10315:2000), which has been technically revised. It also incorporates the Amendment ISO 10315:2000/Amd 1:2011 and the Technical Corrigendum ISO 10315:2000/Cor 1:2000.

This corrected version of ISO 10315:2013 incorporates the following correction:

— the wording of 4.2 has been clarified.

Introduction

This International Standard 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 cigarette smoke condensates. The set comprises:

ISO 3308, ISO 3402, ISO 4387, ISO 8243, ISO 10315, and ISO 10362-1.

A related International Standard, ISO 3400, determines total alkaloids, whereas this International Standard 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.

A bibliography is provided.

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 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 nicotine in smoke condensates — Gas-chromatographic method

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

1   Scope

This International Standard specifies a method for the gas-chromatographic determination of nicotine in cigarette smoke condensates. The smoking of cigarettes and the collection of mainstream smoke are normally carried out in accordance with ISO 4387.

NOTE 1   The method specified in this International Standard is also applicable to the determination of nicotine in cigarette smoke condensates obtained by non-standard smoking.

NOTE 2   In countries not in a position to use the gas-chromatographic method, reference should be made to ISO 3400 for the determination of total nicotine alkaloids. In such cases, values obtained using the method described in ISO 3400 may be used with the addition of a note in the expression of results.

2   Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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   Principle

The smoke condensate from the mainstream smoke is dissolved in a solvent containing an internal standard. The nicotine content of an aliquot of the solution is determined by gas chromatography, and the nicotine content of the whole of the smoke condensate is calculated.

4   Reagents

Use only reagents of recognized analytical reagent grade.

4.1   Carrier gas: helium (CAS: 7440-59-7) or nitrogen (CAS: 7727-37-9) of high purity (at least 99,999 %).

4.2   Auxiliary gases: hydrogen (CAS: 1333-74-0) of high purity (at least 99,999 %) and air for the flame ionization detector.

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

4.4 **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 on samples 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 9).

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

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

Store this at 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.

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

4.7 **Calibration solutions**

Dissolve the nicotine (4.6) in the solvent (4.5) 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 (22 ± 2) °C before use.

5 Apparatus

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

5.1 **Gas-chromatograph**, equipped with a flame ionization detector, recorder, and integrator or other suitable data handling instrument (see Clause 9).

5.2 **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 9).

6 Procedure

6.1 Test portion

Prepare the test portion by dissolving the smoke condensate obtained by the machine smoking of a known number of cigarettes in a fixed volume of the solvent (4.5) 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 6.3) provided that there is adequate volume for effective extraction of the smoke condensate. Analysis should be performed as soon as possible, but if storage is inevitable then store the sample at between 0 °C and 4 °C and exclude light. For standard smoking, refer to ISO 4387.

6.2 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, 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 9).

Suitable operating conditions are as follows:

— column temperature, 170 °C (isothermal);

— injection temperature, 250 °C;

— detector temperature, 250 °C;

— carrier gas, helium, or nitrogen 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 9).

6.3 Calibration of the gas chromatograph

Inject an aliquot (2 µl) of each of the calibration solutions (4.7) into the gas chromatograph. Record the peak areas (or heights) of the nicotine and internal standard (4.4). 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 nicotine concentrations in accordance with the area ratios, and calculate a linear regression equation (concentration of nicotine according to the area ratios) from these data. The graph should be linear and the regression line should pass through the origin. Use the slope of the regression equation.

Perform this full calibration procedure daily. In addition, inject an aliquot of an intermediate concentration standard 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.

6.4 Determination

Inject aliquots (2 µl) of the test portion (see 6.1) 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 6.1).

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 the smoke traps is considered adequate.

7 Expression of results

Calculate the concentration of nicotine in the test portion using the graph or linear regression equation prepared in 6.3. From the concentration of nicotine in the test portion, calculate the amount of nicotine in the smoke condensate. Deduce the amount in the cigarettes smoked. Express the test results in milligrams per cigarette, m_N, for each channel to the nearest 0,01 mg and the average per cigarette to the nearest 0,1 mg.

8 Repeatability and reproducibility

A major international collaborative study involving 30 laboratories and 6 samples, conducted in 1990, showed that when cigarettes are smoked in accordance with ISO 4387 and the resulting smoke solutions