INTERNATIONAL STANDARD

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

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

This second edition cancels and replaces the first edition (ISO 10315:1991), which has been technically revised.

Annex A of this International Standard is for information only.

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.

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

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. However, 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 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 normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 13276, *Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid*.

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 or nitrogen of high purity.

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

4.3 **Propan-2-ol**, with maximum water content of 1,0 mg/ml.

4.4 **Internal standard**: *n*-heptadecane or quinaldine (of purity at least 99 %).

Carvone, *n*-octadecane 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 of known purity and verified in accordance with ISO 13276.

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

Nicotine salicylate of known purity and verified in accordance with ISO 13276 may also be used.

4.7 Calibration solutions

Dissolve the nicotine (4.6) in the solvent (4.5) to produce a series of at least four calibration solutions whose concentrations 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 % 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 or 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 (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 (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 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
0,091	0,040	0,069
0,179	0,046	0,069
0,326	0,050	0,076
0,673	0,077	0,109
0,835	0,079	0,142
1,412	0,107	0,195

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 CORESTA Report 91/1.

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

9 Alternative gas chromatographic procedures and analysis precautions

9.1 General

Alternative gas chromatographic columns, both packed and capillary have been found suitable for the determination of nicotine in smoke condensate. 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.

The data in clause 8 refer to the reference column; appropriate data for these alternative procedures are not yet available.

9.2 Alternative columns

9.2.1 Packed columns

The following may be used as alternative stationary phases in the column described in 5.2:

— 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).

9.2.2 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 poly(ethylene glycol) stationary phases such as CAM (J & W Scientific)[1)], Carbowax-amine (Supelco)[1)], Stabilowax-DB (Restek)[1)], and CP WAX-51 (Chrompack)[1)] give similar data to the PEG 20 000 plus potassium hydroxide packed column in 9.2.1.

9.3 Injection systems

The alternative columns described in 9.2.1 and 9.2.2 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 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 mm/s;

— spilt ratio 20:1.

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

9.4 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 on samples 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.

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

10 Test report

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

Annex A
(informative)

Use of this method with the gas-chromatographic determination of water

This method may be used in conjunction with, or simultaneously with, the gas-chromatographic method of water determination in smoke condensates specified in ISO 10362-1. This may be carried out by

— the addition of an appropriate quantity of the internal standard specified for the water determination in the solvent described in 4.5;

— the use of helium, preferably, as the carrier gas;

— injection of an aliquot of the smoke condensate solution onto a column for water analysis, which is connected to a thermal conductivity detector, as well as onto the nicotine column and detector described in this method.

A simultaneous automated analysis of nicotine and water may be achieved by using a splitting system or an auto-sampler with two injection positions. When determining nicotine and water from the same sample sequentially, the water determination is performed first to prevent absorption of water by the sample affecting the final result.