INTERNATIONAL STANDARD

ISO 10315

First edition
1991-08-01

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

Reference number
ISO 10315:1991(E)

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.

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.

International Standard ISO 10315 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, in collaboration with the Cooperation Centre for Scientific Research Relating to Tobacco (CORESTA).

Annexes A and B of this International Standard are for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

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 condensate. The set comprises

ISO 3308:1991[1)], *Routine analytical cigarette-smoking machine — Definitions and standard conditions.*

ISO 3402-1991[1)], *Tobacco and tobacco products — Atmosphere for conditioning and testing.*

ISO 4387:1991[1)], *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine.*

ISO 8243:1991[1)], *Cigarettes — Sampling.*

ISO 10315:1991, *Cigarettes — Determination of nicotine in smoke condensates — Gas-chromatographic method.*

ISO 10362-1:1991, *Cigarettes — Determination of water in smoke condensates — Part 1: Gas-chromatographic method.*

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

Annex B refers to ISO 3400, which determines total alkaloids, whereas ISO 10315 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.

1) To be published.

This page intentionally left blank

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 collection of mainstream smoke are normally carried out in accordance with ISO 4387. However, the method is also applicable to the determination of nicotine in cigarette smoke condensates obtained by non-standard smoking.

NOTE 1 In the countries that are 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 an additional note in the expression of results.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 4387:1991[2], *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine.*

ISO 8243:1991[2], *Cigarettes — Sampling.*

3 Principle

Dissolution of the smoke condensate from the mainstream smoke in a solvent. Determination of the nicotine content of an aliquot of the solution by gas chromatography and calculation of the nicotine content of the whole of the smoke condensate.

4 Reagents

All reagents shall be of recognized analytical reagent grade.

4.1 Carrier gas.

Helium or nitrogen.

4.2 Auxiliary gases.

Air and hydrogen of high purity for the flame ionization detector.

4.3 Propan-2-ol.

Maximum water content: 1,0 mg per cm^3.

4.4 Internal standard.

n-Heptadecane or quinaldine (of purity at least 99 %).

4.5 Extraction solvent.

Propan-2-ol (4.3) containing 0,5 g per litre of internal standard (4.4).

4.6 Reference substance.

Nicotine (of purity at least 98 %). Store at 0 °C to + 4 °C and exclude light.

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 per

2) To be published.

cm^3 to 2,0 mg per cm^3). Store these solutions at 0 °C to + 4 °C and exclude light.

5 Apparatus

Usual laboratory apparatus and the following items:

5.1 Gas-chromatograph, equipped with flame ionization detector and recorder or integrator.

5.2 Column, of internal diameter between 2 mm and 4 mm and preferably of length 1,5 m to 2 m. Stationary phase: 10 % PEG 20 000 plus 2 % potassium hydroxide on an acid washed silanized support material, 80 mesh to 100 mesh.

NOTES

2 The column is preferably made of glass but other materials such as deactivated stainless steel or nickel may be used. Alternative stationary phases, such as 2 % Versamid 900[3] plus 1 % potassium hydroxide or lower loadings of PEG 20 000 with or without potassium hydroxide, may be used. If alternative stationary phases are used it is necessary to ensure that the peak due to nicotine is well resolved from peaks due to other smoke components, the internal standard and the solvent.

3 Other columns have been proposed and are being studied by CORESTA.

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 20 cm^3 for 44 mm disc or 50 cm^3 for 92 mm disc of the solvent (4.5) to obtain the concentration of nicotine adjusted to the calibration graph (6.3). Analysis should be performed as soon as possible but if storage is inevitable then store the sample at 0 °C to 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, recorder or integrator and autosampler (if one is used) in accordance with the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, nicotine and other smoke component peaks, especially neophytadiene, are well resolved.

Suitable operating conditions are

— 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 cm^3 per min

— Injection volume: 2µl

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

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. 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 according to 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 to the 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.

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

3) Trade name. Versamid 900 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

results in milligrams per cigarette 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 repeatability (r) and reproducibility (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 value (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 value (R) on average not more than once in 20 cases in the normal and correct operation of the method.

Data analysis gave the estimates as summarized in table 1.

Table 1

Mean yield of nicotine mg	Repeatability conditions r	Reproducibility conditions R
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 Test report

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

Annex A
(informative)

Use of this method in conjunction, or simultaneously with the gas-chromatographic determination of water

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

a) the addition of an appropriate quantity of the internal standard prescribed for the water determination in the solvent described in 4.5;

b) the use, preferably of helium, as the carrier gas;

c) an injection of an aliquot of the smoke condensate solution on to a column for water analysis, which is connected to a thermal conductivity detector, as well as on to 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.

Annex B
(informative)

Bibliography

[1] ISO 3400:1989, *Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method.*