INTERNATIONAL
STANDARD

ISO
3400

Third edition
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Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method

Cigarettes — Détermination des alcaloïdes dans les condensats de fumée — Méthode spectrométrique

Reference number
ISO 3400:1997(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 3400 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products.*

This third edition cancels and replaces the second edition (ISO 3400:1989), which has been technically revised.

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

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Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method

1 Scope

This International Standard specifies a method for the spectrometric determination of alkaloids in cigarette smoke condensates. The smoking of cigarettes and collection of mainstream smoke are normally carried out in accordance with ISO 4387. However, this method is also applicable to the determination of nicotine in cigarette smoke condensates obtained by non-standard smoking.

> NOTE 1 ISO 4387 specifies the use of gas-chromatography for the determination of nicotine in smoke condensate solutions (see also ISO 10315). In countries that are not in a position to use the gas-chromatographic method, the determination of nicotine in smoke condensate should be performed by the method described in this International Standard and an appropriate note made in the expression of results.

This International Standard 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.

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 listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

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

ISO 13276:1997, *Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid.*

3 Principle

Dissolution of the smoke condensate from the mainstream smoke in a solvent. Steam distillation of an aliquot portion of the solution in two steps. Acidification of the solution with a mineral acid and removal of neutral and acid steam-volatile substances by distillation followed by making the same solution strongly alkaline and distillation of the alkaloids. Spectrometric measurement of the absorbance of the distillate from the alkaline distillation and calculation of the alkaloid content expressed as nicotine.

4 Reagents

Use only reagents of recognized analytical grade and water of grade 3 in accordance with ISO 3696.

4.1 Propan-2-ol [$(CH_3)_2CHOH$], extraction solvent.

4.2 Sodium hydroxide solution, $c(\text{NaOH}) = 8$ mol/l.

4.3 Sulfuric acid solution, $c(H_2SO_4) = 1$ mol/l.

4.4 Sulfuric acid solution, $c(H_2SO_4) = 0,025$ mol/l.

4.5 Nicotine, minimum purity 98 %.

Store at 0 °C to +4 °C and exclude light.
Test the nicotine purity in accordance with ISO 13276.

NOTE 2 Nicotine hydrogen tartrate, minimum purity 98 %, may also be used.

5 Apparatus

Usual laboratory apparatus and the following items.

5.1 Steam distillation apparatus, consisting of the parts listed in 5.1.1 to 5.1.4.

It is essential to check the performance of the steam distillation apparatus regularly. Prepare a pure nicotine solution using the extraction solvent (4.1) such that a 10 ml aliquot portion contains an accurately known amount of nicotine at a level of approximately 5 mg. Use this as a test portion (6.2) and ensure that the recovery is better than 98 %. If not, optimize by modification of the distillation rate.

NOTE 3 The diagrams of apparatus currently used (annex A, figures A.1 to A.3) are given as examples. Other apparatus may also be used provided that the results obtained are the same.

5.1.1 Distillation chamber

A cylindrical, vertically mounted distillation chamber of about 50 ml to 100 ml capacity, which has a steam inlet at its base.

It shall be possible to heat the chamber in order to maintain a constant liquid level during the distillation.

5.1.2 Distillation splash head.

5.1.3 Jacketed coil condenser, with spherical joint fitting on to the distillation splash head (5.1.2).

5.1.4 Plug-type funnel, or other system for addition of sodium hydroxide solution.

It is important that during the introduction of the sodium hydroxide solution (4.2) steam does not escape through the tap or stopper.

5.2 Spectrometer, covering a wavelength range from 230 nm to 290 nm. (See also Note 7).

5.3 Matched quartz cells, having an optical path length of 1 cm.

Larger matched quartz cells may be used for very low concentrations of nicotine.

5.4 Volumetric flasks, of capacity 250 ml, with ground glass stoppers.

5.5 One-mark pipettes, of capacities 5 ml, 10 ml and 25 ml.

5.6 Glass funnels, of diameter about 55 mm.

5.7 Filter paper, fast-filtering grade, of a diameter to suit the glass funnel (5.6).

6 Procedure

6.1 Preparation of test sample

Prepare the test sample by dissolving the smoke condensate obtained by machine-smoking a known number of cigarettes. For the 44 mm disc, use 25 ml of the solvent (4.1); for the 92 mm disc, use 50 ml of the solvent. 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 and dissolving of smoke condensate, refer to ISO 4387.

6.2 Test portion

Take, as the test portion, an aliquot portion (V_1) of the smoke condensate solution corresponding to about 3 mg to 5 mg of alkaloid.

NOTE 4 This specification cannot be met for low nicotine cigarettes.

6.3 Distillation

WARNING - Take care during the distillation while sodium hydroxide is being added and wear appropriate safety equipment.

NOTE 5 The quantities specified in this subclause relate to the type of apparatus specified in 5.1. If other apparatus is used, these quantities may be modified provided that the results obtained are the same.

Introduce the test portion (6.2) into the distillation chamber of the distillation apparatus (5.1). Add 10 ml of the sulfuric acid solution (4.3) and start the distillation. Collect about 100 ml of the distillate in a beaker. Without stopping the distillation, remove the beaker and discard the distillate. Insert the delivery tube into a 250 ml volumetric flask (5.4) containing 10 ml of the sulfuric acid solution (4.3). Ensure that the end of the tube is immersed in the acid. Slowly add 5 ml of the sodium hydroxide solution (4.2), closing the inlet funnel as the last drops pass through. Collect 220 ml to 230 ml of distillate. Remove the flask whilst rinsing the delivery tube with a little water. Terminate the distillation and rinse the still. Ensuring that the flask is at room temperature, dilute to the mark (volume V_2) with water. Mix and use this solution to determine spectrometrically the alkaloids in the smoke condensate. Filter if the solution is not clear.

NOTE 6 If filtration is necessary, either the first 150 ml of filtrate should be discarded or, before use, the filter paper should be washed with a sufficient amount of water and then dried.

6.4 Determination of alkaloids in the distillate

Using the spectrometer (5.2), measure the absorbance of the filtrate at wavelengths of 236 nm, 259 nm and 282 nm against a reference solution of 10 ml of the sulfuric acid solution (4.3) diluted to 250 ml with water.

If the absorbance at 259 nm exceeds 0,8, dilute an aliquot portion (V_3) of the distillate to a suitable volume (V_4) with the sulfuric acid solution (4.4) and measure the absorbance of this solution as above. The reference solution is also diluted with sulfuric acid solution (4.4) to the same suitable volume (V_4) as the aliquot portion (V_3). If the absorbance at 259 nm is less than 0,2 use larger cells (5.3).

Carry out two determinations of the same test sample under identical conditions.

7 Calculation and expression of results

The alkaloid content H_{nic} of the smoke condensate, expressed as nicotine in milligrams per cigarette, for each smoking run is given by the formula

$$H_{\text{nic}} = \frac{A V_0 V_2 V_4}{a\, l\, V_1 V_3\, n} \qquad (1)$$

where

a is the absorptivity (decadic extinction coefficient) of nicotine in 0,025 mol/l sulfuric acid solution (4.4) (i. e. 34,3 at the absorption maximum of 259 nm) given in $\text{ml}\cdot\text{mg}^{-1}\cdot\text{cm}^{-1}$;

A is the corrected absorbance (extinction) calculated from the absorbances measured at wavelengths of 236 nm, 259 nm and 282 nm:

$$A = 1{,}059\left(A_{259} - \frac{A_{236} + A_{282}}{2}\right) \qquad (2)$$

l is the optical path length of the cell, in centimetres;

V_0 is the volume of propan-2-ol solution of crude smoke condensate, in millilitres;

V_1 is the aliquot portion of V_0 used for the distillation, in millilitres;

V_2 is the volume of distillate from the alkaline distillation, in millilitres;

V_3 is the aliquot portion of distillate V_2 used for further dilution to V_4, in millilitres;

V_4 is the volume to which the aliquot portion V_3 of the distillate was further diluted, in millilitres;

n is the number of cigarettes smoked into the smoking trap.

NOTE 7 Experience has shown that the figure for the nicotine extinction coefficient, i. e. 34,3 and the factor of 1,059 in the corrected absorbance equation established in reference [2] (annex B) does not apply to all spectrometers. Every spectrometer should, therefore, be checked to ensure that these factors are valid using a series of standard nicotine solutions in the working range.

Express the test results as follows:

a) alkaloid content, expressed as nicotine, in milligrams per cigarette for each channel, to the nearest 0,01 mg;

b) alkaloid content, expressed as nicotine, as the average per cigarette, to the nearest 0,1 mg.

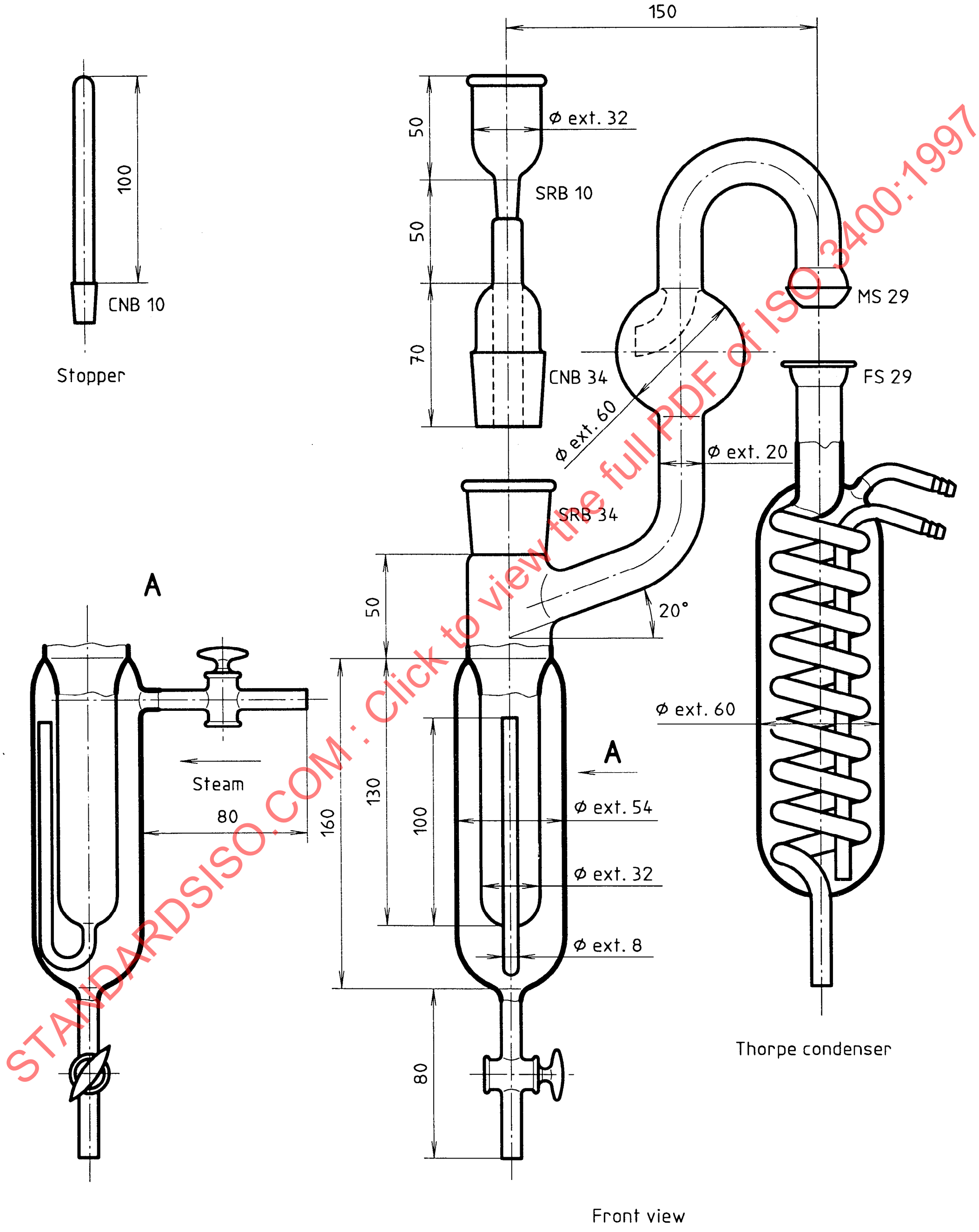
8 Test report

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 analysis). It shall also give all details necessary for the identification of the cigarette smoked.

Annex A
(informative)

Diagrams of apparatus currently used

Dimensions in millimetres



NOTES

1 All glass is medium wall borosilicate

2 Stopcocks: 4 mm bore PTFE

Figure A.1 - Example of apparatus currently in use

Dimensions in millimetres

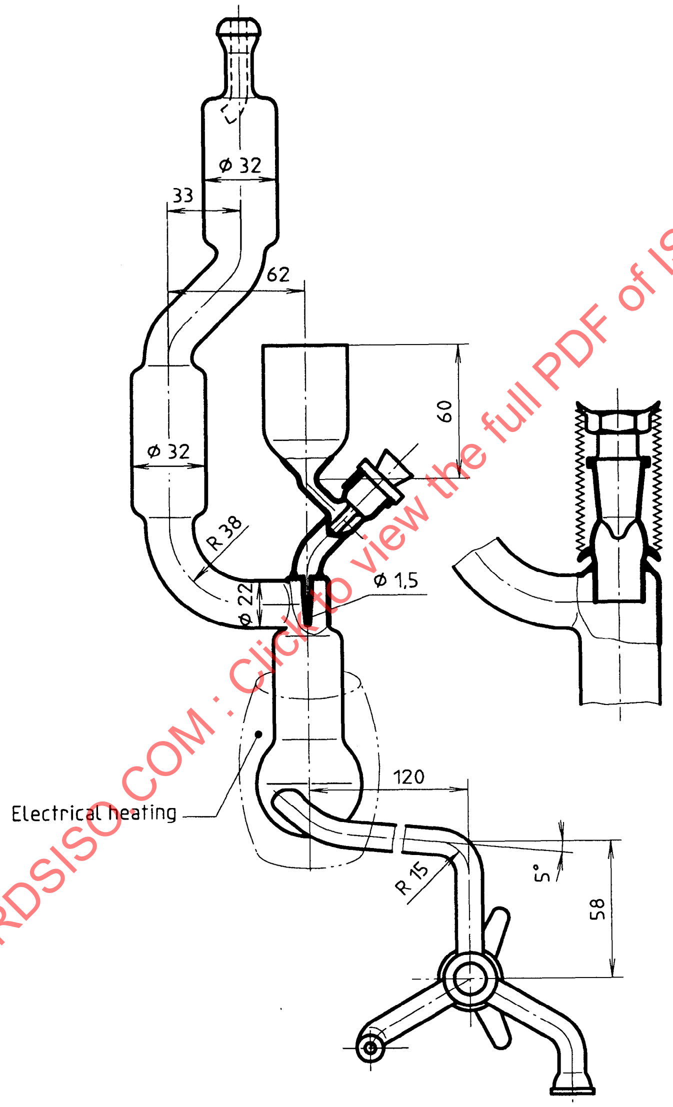


Figure A.2 - Example of apparatus currently in use