भारतीय मानक
Indian Standard

IS 16133 : 2018
ISO 3401 : 1991

सिगरेट — फिल्टर द्वारा एल्कालायड प्रतिधारण ज्ञात करना — स्पेक्ट्रोमीटरी पद्धति

Cigarettes — Determination of Alkaloid Retention by the Filters — Spectrometric Method

ICS 65.160

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July 2018 **Price Group 5**

Tobacco and Tobacco Products Sectional Committee, FAD 04

NATIONAL FOREWORD

This Indian Standard which is identical with ISO 3401 : 1991 'Cigarettes — Determination of alkaloid retention by the filters — Spectrometric method' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Tobacco and Tobacco Products Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are however not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards, which are to be substituted in their places, are listed below along with their degree of equivalence for the editions indicated:

	International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
1)	ISO 3308 : 1991 Routine analytical cigarette-smoking machine — Definitions and standard conditions	IS 16022 : 2012 Routine analytical cigarette-Smoking machine — Definitions and standard conditions	Identical with ISO 3308 : 2000
2)	ISO 3400 : 1989 Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method	IS 16134 : 2014 Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method	Identical with ISO 3400 : 1997
	ISO 3402 : 1999 Tobacco and tobacco products — Atmosphere for conditioning and testing	IS 16121 : 2013 Tobacco and tobacco products — Atmosphere for conditioning and testing	Identical
3)	ISO 4387 : 1991 Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine	IS 16023 : 2012 Cigarettes Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine	Identical with ISO 4387 : 2000
4)	ISO 8243 : 1981 Cigarettes — Sampling	IS 12942 : 2008 Cigarettes — Sampling	Identical with ISO 8243 : 2006

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

CIGARETTES — DETERMINATION OF ALKALOID RETENTION BY THE FILTERS — SPECTROMETRIC METHOD

1 Scope

This International Standard specifies two methods for the spectrometric determination of alkaloid retention by filters of cigarettes:

— the direct method;

— the indirect method.

The methods are applicable to filter cigarettes. The direct method shall be used unless it is not applicable owing to incomplete recovery of the retained alkaloids from the filter material (for example, with some types of charcoal filters). The indirect method is not applicable to cigarettes with perforated or porous filter tipping wraps. This International Standard is not applicable in the case of filters having an irreversible nicotine retention and equipped with perforated or porous wrapping.

NOTE 1 These methods determine the retention only of alkaloids of tobacco smoke, expressed as nicotine. The retention of other substances present in the mainstream smoke is not necessarily related to the alkaloid retention.

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 3308:1991, *Routine analytical cigarette-smoking machine — Definitions and standard conditions.*

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

ISO 3402:1991, *Tobacco and tobacco products — Atmosphere for conditioning and testing.*

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

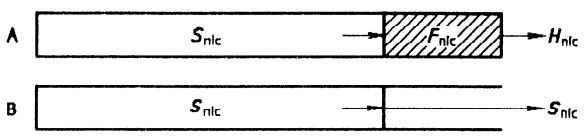
ISO 8243:1991, *Cigarettes — Sampling.*

3 Definition

For the purposes of this International Standard, the following definition applies.

3.1 alkaloid retention index R_{nic} of a cigarette filter: The ratio, expressed as a percentage by mass, of the alkaloids retained by the filter to those entering the filter:

$$R_{nic} = \frac{F_{nic}}{S_{nic}} \times 100$$



The alkaloid retention index may be determined as follows:

a) *Directly*, by measurement of the mass of alkaloids retained by the filter and of the mass of alkaloids in the mainstream smoke leaving the filter:

$$R_{nic} = \frac{F_{nic}}{H_{nic} + F_{nic}} \times 100 = \frac{F_{nic}}{S_{nic}} \times 100$$

where

F_{nic} is the mass of alkaloids retained by the filter;

H_{nic} is the mass of alkaloids in the mainstream smoke leaving the filter;

S_{nic} is the mass of alkaloids entering the filter [see (**A**) above].

b) *Indirectly*, by measurement of the difference between the mass of alkaloids contained in the mainstream smoke from a cigarette with filter (**A**) and of the corresponding mass from another cigarette with filter material removed (**B**), the smoked length of which is the same as that of the filter cigarette (**A**):

$$R_{nic} = \frac{S_{nic} - H_{nic}}{S_{nic}} \times 100 = \frac{F_{nic}}{S_{nic}} \times 100$$

where

H_{nic} is the mass of alkaloids in the mainstream smoke from the cigarette with filter (**A**):

S_{nic} is the mass of alkaloids in the mainstream smoke from the cigarette with the filter material removed (**B**).

4 Principle

4.1 Direct method

Smoking of the filter cigarettes (**A**), in accordance with ISO 4387 on a routine analytical cigarette-smoking machine complying with the requirements of ISO 3308, and removal of the filter tips from the cigarette butts remaining; subjection of the filter tips, after addition of methanol, to steam distillation from acid solution to remove neutral and acid steam-volatile substances, and discarding of the distillate.

Rendering of the residue in the distillation chamber alkaline by addition of alkali, and steam distillation of the alkaloids; estimation of the alkaloid content by spectrometric measurement of the absorbance of the distillate from the alkaline distillation, and calculation of the alkaloid content as nicotine.

Collection of the mainstream smoke condensate from the filter cigarettes (**A**), preparation of a methanolic solution of the condensate and determination of its alkaloid content by distillation in accordance with ISO 3400.

4.2 Indirect method

4.2.1 Smoking of the filter cigarettes (**A**) in accordance with ISO 4387 on a routine analytical cigarette-smoking machine complying with the requirements of ISO 3308, collection of the mainstream smoke condensate, preparation of a methanolic solution of the condensate and determination of its alkaloid content by distillation in accordance with ISO 3400.

4.2.2 Removal of the filter material from a second sample of identical filter cigarettes (**A**), smoking of the remaining tobacco rods (**B**) in accordance with ISO 4387 on a routine analytical cigarette-smoking machine complying with the requirements of ISO 3308, collection of the mainstream smoke condensate, preparation of a methanolic solution of the condensate and determination of its alkaloid content by distillation in accordance with ISO 3400.

5 Reagents

Use only reagents of recognized analytical grade and distilled water or water of at least equivalent purity.

5.1 Methanol.

5.2 Sodium hydroxide, solution, $c(NaOH)$ = 8 mol/l.

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

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

5.5 Nicotine, minimum purity 98 %.

6 Apparatus

Usual laboratory apparatus and the following items:

6.1 Conditioning enclosure, regulated in accordance with the requirements of ISO 3402.

6.2 Routine analytical cigarette-smoking machine, complying with the requirements of ISO 3308, with glass fibre filter smoke trap (see ISO 4387).

6.3 Steam distillation apparatus, consisting of the following parts:

6.3.1 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.

6.3.2 Distillation splash head.

6.3.3 Jacketed coil condenser, with spherical joint fitting on to the distillation splash head (6.3.2).

6.3.4 Plug-type funnel, or other system for addition of sodium hydroxide solution and, if required, the filter tips.

6.3.5 Testing of the distillation apparatus.

Test the system in accordance with the indicated procedure (ISO 3400) with pure nicotine solution (5.5) at the maximum expected level. Recovery shall be at least 98 % of the theoretical value. If not, optimize by modification of the distillation rate. For routine tests it is possible to use nicotine salt calibrated against pure nicotine (5.5).

NOTE 2 The diagrams of apparatus currently used (figures 1 to 3) are given as examples. Other apparatus may also be used provided that the results obtained are the same.

6.4 Spectrometer, covering a wavelength range from 230 nm to 290 nm.

6.5 Quartz cells, having an optical path length of 1 cm, or identical matched cells in the case of a single beam apparatus.

The absorbance of the cells shall be equal before and after each measurement; if not, a suitable correction shall be applied.

6.6 Volumetric flasks, of capacity 250 ml, with ground stoppers.

6.7 One-mark pipettes, of capacities 5 ml, 10 ml or 25 ml.

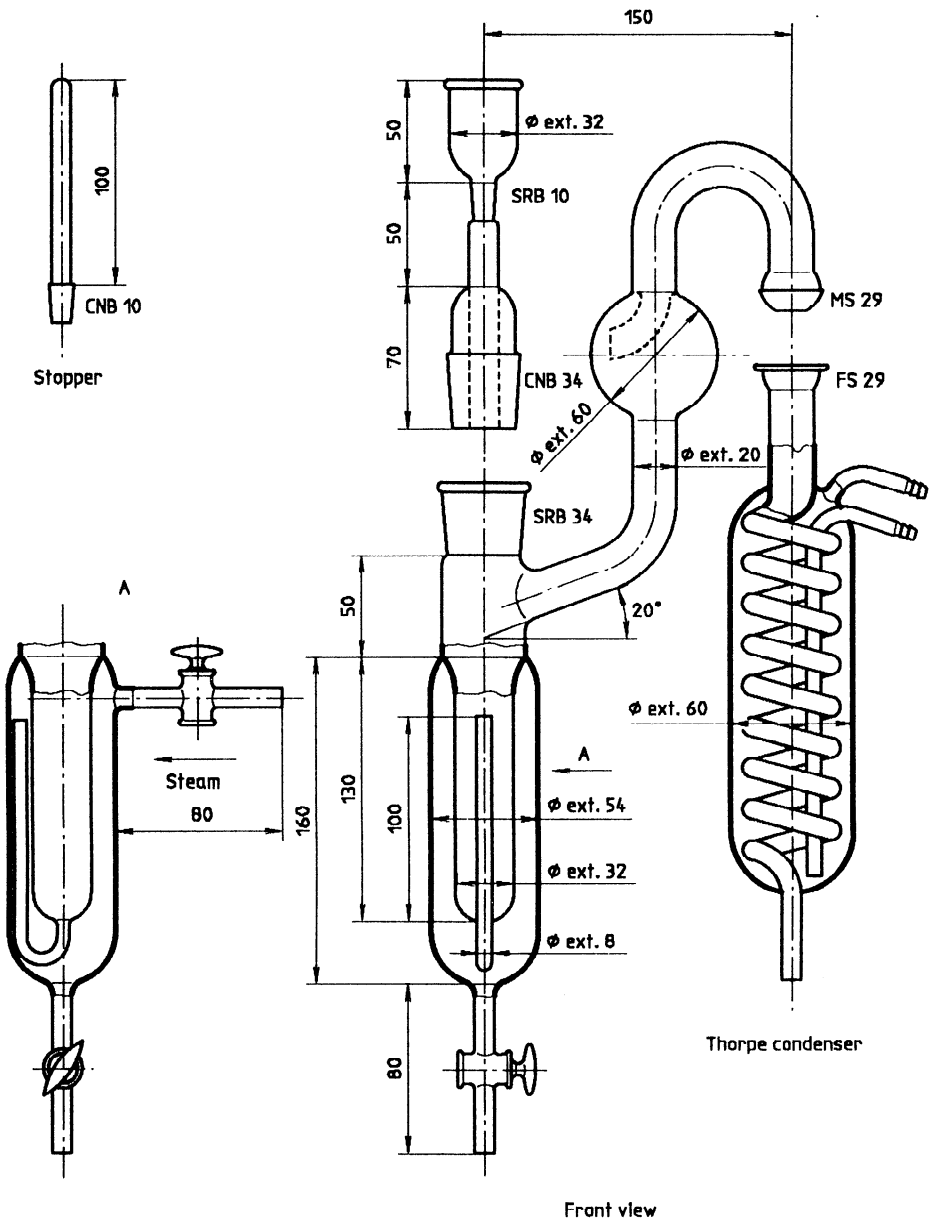
6.8 Glass funnels, of diameter about 55 mm.

6.9 Filter paper, fast filtering grade.

7 Sampling

Carry out sampling in accordance with the method specified in ISO 8243.

Dimensions in millimetres



NOTES

1 All glass is medium wall borosilicate.

2 Stopcocks: 4 mm bore PTFE.

Figure 1 — Example of apparatus currently in use

Dimensions in millimetres

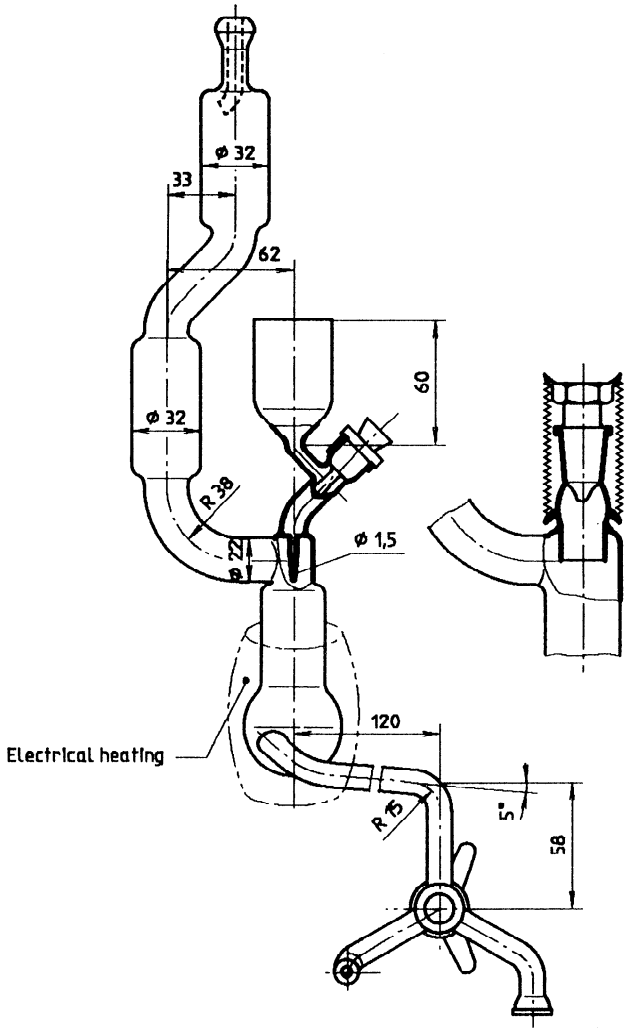
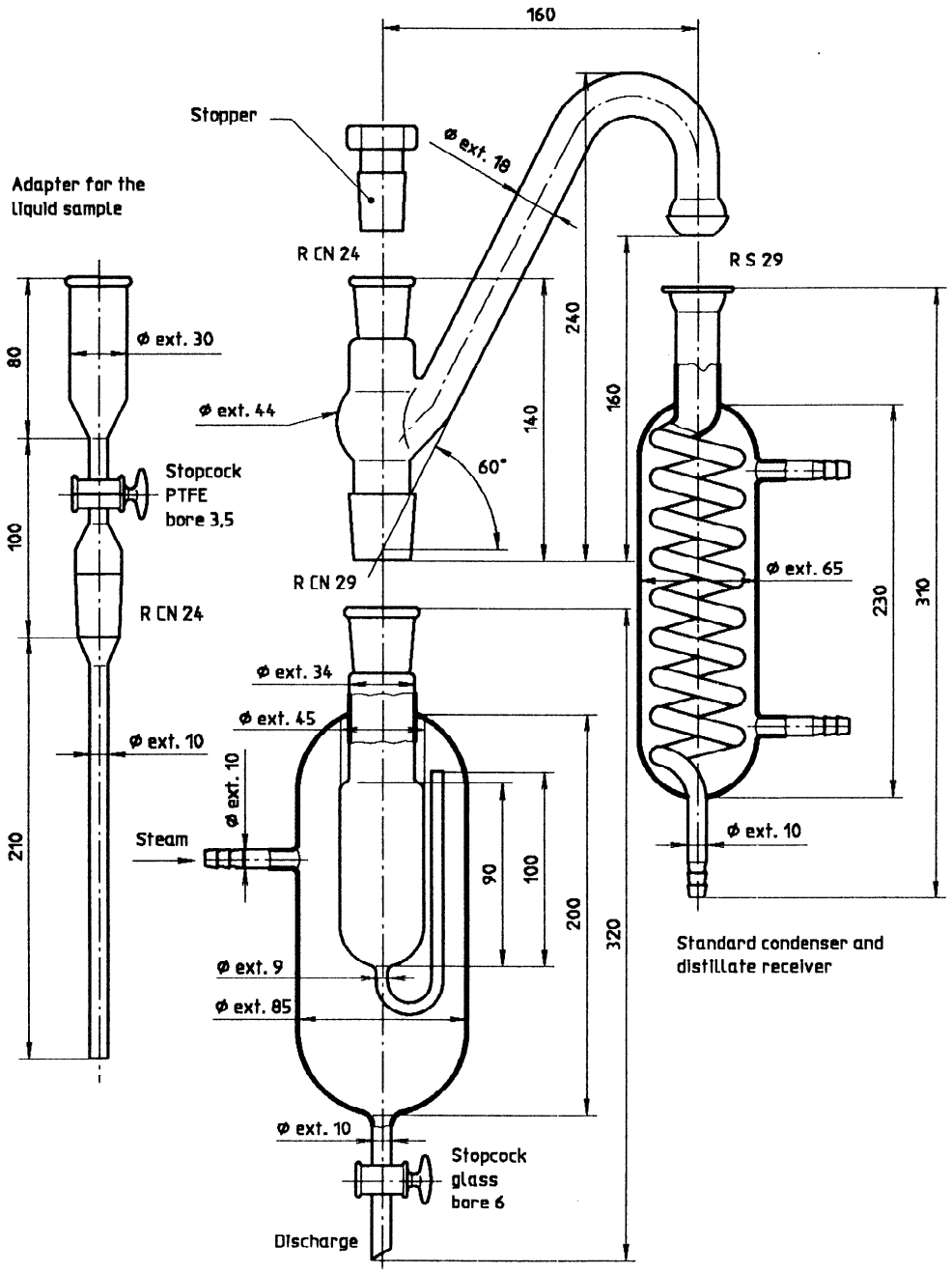


Figure 2 — Example of apparatus currently in use



NOTE — All glass is medium wall borosilicate.

Figure 3 — Example of apparatus currently in use

8 Procedure

8.1 Direct method

8.1.1 Preparation of test sample

Select from the laboratory sample prepared in accordance with clause 7 the required number of filter cigarettes for the set of smoking runs to be carried out and condition them in accordance with ISO 3402 in the conditioning enclosure (6.1).

8.1.2 Determination

8.1.2.1 Smoking

Smoke the conditioned cigarettes (8.1.1) in accordance with ISO 4387 on the routine analytical cigarette-smoking machine (6.2) in one or more smoking processes. Collect the mainstream smoke condensate in the appropriate trap for each smoking process. As soon as each cigarette has been smoked to the required butt length, extinguish it.

At the end of each smoking process remove the filter tips and the traps. Proceed to 8.1.2.2 and 8.1.2.3 without delay at any stage.

8.1.2.2 Determination of alkaloids retained in the cigarette filters (F_{nic})

Carefully remove any adhering tobacco from the filter tips and cut them open. Open the still and place a maximum of three tips in the distillation chamber. Close the still and introduce about 10 ml of the methanol (5.1) into the chamber.

NOTE 3 A less desirable alternative to placing the tips directly into the distillation chamber is to use a methanolic extract, as follows: Place 20 tips and 100 ml of methanol in a flask. Shake it for 30 min or allow it to stand overnight and shake by hand. Using a pipette, transfer 10 ml of extract to the distillation chamber.

WARNING — Take care during the distillation while sodium hydroxide is being added.

Add 10 ml of the sulfuric acid solution (5.3) 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 (6.6) containing 10 ml of the sulfuric acid solution (5.3). Ensure that the end of the tube is immersed in the acid. Slowly and carefully add 5 ml of the sodium hydroxide solution (5.2) to the distillation chamber, closing the inlet funnel (6.3.4) 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 clean the still, using forceps if necessary to remove the filter tips.

Ensure that the flask is at room temperature, dilute the distillate to the mark with water and mix. If the solution is not clear, filter through a filter paper (6.9), discarding the first 150 ml of filtrate. Use the solution to determine spectrometrically the alkaloids retained in the cigarette filters, in accordance with ISO 3400.

8.1.2.3 Determination of alkaloids in the mainstream smoke condensate (H_{nic})

Having removed the traps containing the mainstream smoke condensate from the filter cigarettes smoked, proceed according to the following method:

Dismantle the holder and remove any sealing ring with forceps. Remove the disc with forceps and fold twice. Place the folded disc in a conical flask of capacity 150 ml containing a suitable volume of methanol (5.1). The volume shall be adjusted according to the number of traps and cigarettes smoked so that the alkaloids from two to three cigarettes are contained in the aliquot of methanolic solution taken for the distillation (ideally 20 ml).

Wipe the inner surface of the holder front with two quarters of a blank filter disc held with forceps, and put these into the flask. Close the flask and shake on an electrical shaker for 20 min or let it stand for 16 h ensuring that the disc does not disintegrate. Use, as the test portion, an aliquot of this solution to determine the alkaloids in the mainstream smoke condensate in accordance with ISO 3400.

8.2 Indirect method

8.2.1 Preparation of test sample

Select from the laboratory sample prepared in accordance with clause 7 twice the number of filter cigarettes required for the direct method and condition them in accordance with ISO 3402 in the conditioning enclosure (6.1). Separate the total prepared sample into two identical sub-samples, X and Y.

Remove the filter material of the cigarettes from sub-sample Y, leaving the tipping sleeve in place. If the tipping sleeve has to be removed, replace it with a new tipping sleeve of the same length as the original tipping on the cigarette.

8.2.2 Determination

8.2.2.1 Determination of alkaloids in the mainstream smoke condensate of the filter cigarettes of sub-sample X (H_{nic})

Smoke the cigarettes of sub-sample X in accordance with ISO 4387 on the routine analytical cigarette-smoking machine (6.2) to the required butt length in one or more smoking runs and collect the main-

stream smoke condensate in a trap for each smoking run.

Remove the traps and continue the determination in accordance with 8.1.2.3 .

8.2.2.2 Determination of alkaloids in the mainstream smoke condensate of the non-filter cigarettes of sub-sample Y (S_{nic})

Smoke the cigarettes of sub-sample Y in the same way as those of sub-sample X, ensuring that the length of the cigarette projecting from the cigarette holder of the smoking machine is the same in both sub-samples and that the length of tobacco rod smoked is the same for both sub-samples.

Collect the mainstream smoke condensate and prepare a methanolic solution of the smoke condensate as described in 8.2.2.1, separating and adding the tipping sleeve to the methanolic solution. Determine the alkaloid content of the smoke condensate solution as described in 8.2.2.1.

9 Calculation and expression of results

9.1 Method of calculation and formulae

9.1.1 Calculate the alkaloid content of the mainstream smoke condensate, expressed in milligrams per cigarette, for each individual smoking run, in accordance with ISO 3400.

9.1.2 Calculate the mean alkaloid content of the mainstream smoke condensate per set of smoking runs as the mean of the results obtained for each set of smoking runs in accordance with ISO 3400.

9.1.3 Calculate the mean alkaloid content of the mainstream smoke condensate for the whole test sample as the mean of the results obtained for each smoking run.

9.1.4 Calculate the alkaloid content of the smoke condensate retained by the filter, in milligrams per cigarette filter, for each smoking run in accordance with ISO 3400.

9.1.5 Calculate the mean alkaloid content of the smoke condensate retained by the filter for the whole of the filters of the test sample as the mean of the results obtained for each smoking run.

9.1.6 The mean alkaloid retention index R_{nic}, expressed as a percentage by mass, of all the filters of the test sample is equal to

9.1.6.1 Direct method

$$R_{nic} = \frac{F_{nic}}{H_{nic} + F_{nic}} \times 100$$

9.1.6.2 Indirect method

$$R_{nic} = \frac{S_{nic} - H_{nic}}{S_{nic}} \times 100$$

9.2 Expression of results

Express the test results as follows:

a) alkaloid content, expressed as nicotine, of the mainstream smoke condensate, in milligrams per cigarette smoked, to the nearest 0,01 mg for all the filter cigarettes of the test sample;

b) alkaloid content, expressed as nicotine, of the smoke condensate retained by the filter, in milligrams per cigarette smoked, to the nearest 0,01 mg for all the filters of the test sample;

c) mean alkaloid retention index of the test sample of filters, as a percentage, to the nearest 1 %. It is desirable to calculate the confidence interval of the mean alkaloid retention index.

10 Test report

10.1 The test report shall show the method used and the results obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all details required for complete identification of the sample.

The test report shall, in particular, include the items of information listed in 10.2 to 10.5.

10.2 Description of the product tested.

10.3 Sampling procedure:

a) method of sampling;

b) number of cigarettes in the test sample;

c) date and place of purchase or sampling.

10.4 Test conditions, in accordance with ISO 3402.

10.5 Test results, expressed in accordance with 9.2.

10.5.1 If obtained by the direct method:

a) the complete test results;

b) alkaloid content F_{nic}, expressed in milligrams of nicotine per filter, to the nearest 0,01 mg; separate values for each individual smoking run, if available;

c) alkaloid content F_{nic}, expressed in milligrams of nicotine per filter, to the nearest 0,01 mg; separate values for each set of smoking runs;

d) alkaloid content H_{nic}, expressed in milligrams of nicotine per cigarette, of the mainstream smoke condensate, to the nearest 0,01 mg; separate values for each individual smoking run, if available;

e) alkaloid content H_{nic}, expressed in milligrams of nicotine per cigarette, of the mainstream smoke condensate, to the nearest 0,01 mg; separate values for each set of smoking runs;

f) alkaloid retention index R_{nic}, as a percentage, to the nearest 1 %; separate values for each set of smoking runs;

g) date of test;

h) reference to this International Standard.

10.5.2 If obtained by the indirect method:

a) the complete test results;

b) alkaloid content H_{nic}, expressed in milligrams of nicotine per cigarette, of the mainstream smoke condensate from the filter cigarettes, to the nearest 0,01 mg; separate values for each individual smoking run, if available;

c) alkaloid content H_{nic}, expressed in milligrams of nicotine per cigarette, of the mainstream smoke condensate from the filter cigarettes, to the nearest 0,01 mg; separate values for each set of smoking runs;

d) alkaloid content S_{nic}, expressed in milligrams of nicotine per cigarette, of the mainstream smoke condensate from the filterless cigarettes, to the nearest 0,01 mg; separate values for each individual smoking run, if available;

e) alkaloid content S_{nic}, expressed in milligrams of nicotine per cigarette, of the mainstream smoke condensate from the filterless cigarettes, to the nearest 0,01 mg; separate values for each set of smoking runs;

f) alkaloid retention index R_{nic}, as a percentage, to the nearest 1 %; separate values for each set of smoking runs;

g) date of test;

h) reference to this International Standard.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 2016 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

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Amendments Issued Since Publication

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