INTERNATIONAL STANDARD

ISO 15152

First edition
2003-04-15

Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method

Tabac — Détermination de la teneur en alcaloïdes totaux exprimés en nicotine — Méthode par analyse en flux continu

Reference number
ISO 15152:2003(E)

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Foreword

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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 15152 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, Subcommittee SC 2, *Leaf tobacco*.

Introduction

A CORESTA[1)] Task Force studied the various widely used procedures for the determination of total alkaloids in tobacco in order to adopt one of them as the CORESTA Recommended Method. Two procedures were adopted as ISO 2881 and this International Standard. Studies carried out by the CORESTA Task Force between 1989 and 1993 have shown that the two methods may not produce identical results for some dark tobaccos or those containing significant levels of alkaloids other than nicotine. The studies have indicated that these differences may be due to the fact that the recoveries and detection sensitivities of the methods for alkaloids other than nicotine are different.

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1) CORESTA: Cooperation Centre for Scientific Research Relative to Tobacco.

INTERNATIONAL STANDARD **ISO 15152:2003(E)**

Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method

1 Scope

This International Standard specifies a method for the determination of the content of total alkaloids as nicotine in tobacco by continuous-flow analysis.

This method is applicable to manufactured and unmanufactured tobacco.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

3 Principle

An aqueous extract (see below) of the tobacco is prepared and the total alkaloids content (as nicotine) of the extract is determined by reaction with sulfanilic acid and cyanogen chloride. Cyanogen chloride is generated *in situ* by the reaction of potassium cyanide and chloramine T (see Annex A). The developed colour is measured at 460 nm.

Collaborative studies have shown that this method gives equivalent results for water and 5 % acetic acid extracts. It is recommended that 5 % acetic acid extracts should be used if total alkaloids (as nicotine) and reducing substances (see ISO 15153) or reducing carbohydrate analyses (see ISO 15154) are to be carried out simultaneously.

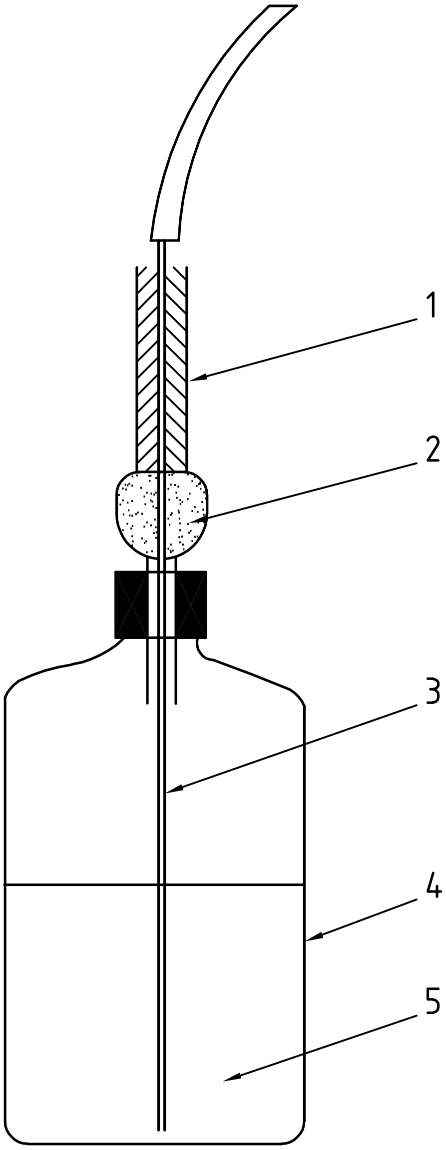
4 Safety precautions

WARNING — Potassium cyanide is poisonous and an irritant, thus all safety precautions shall be observed when handling this material.

Solutions shall be prepared by a designated responsible person. Gloves and safety glasses shall always be used when making up solutions. Bottles of the made-up reagent shall always be carried in a suitable safety carrier. To prevent the escape of vapour into the laboratory, reagent pick-up tubes shall pass through a soda-lime trap into the reagent bottle (see Figure 1).

The cyanide neutralizing solutions A and B (5.5 and 5.6) shall be pumped as shown in the flow diagram (see Figure B.1) and mixed in a 2 litre Büchner flask with magnetic stirring (see Figure 2). All waste solutions containing cyanogen chloride shall be run into this flask where conversion to the “Prussian Blue” complex occurs. The contents of the Büchner flask shall be allowed to overflow into a storage flask, the contents of which shall be stored overnight in a fume cupboard and then disposed of as waste.

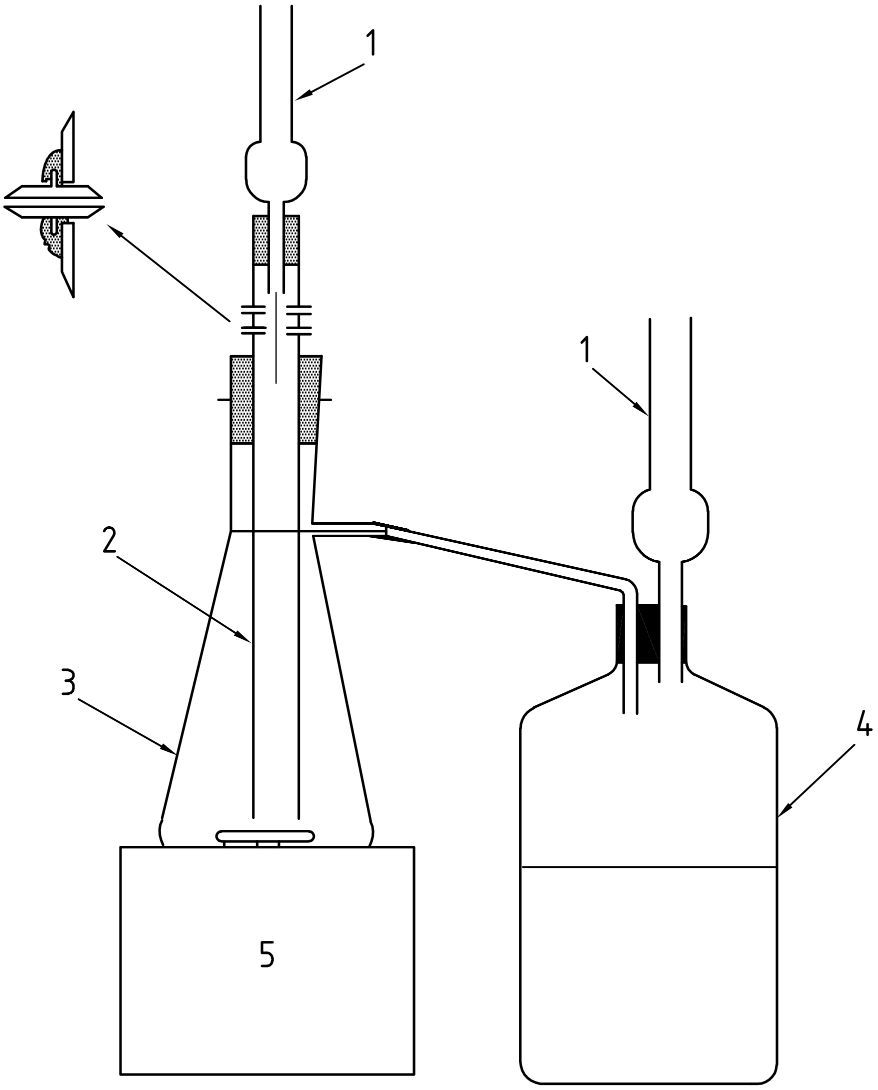
Suitable cyanide poisoning treatment kits are available from laboratory equipment suppliers and shall be located in the vicinity of the analyser to be used by a competent person.



Key

1	soda lime
2	cotton wool
3	reagent pick-up tube
4	storage flask
5	reagent

Figure 1 — Soda-lime trap



Key

1 soda lime traps
2 PVC tube, 2 cm diameter
3 Büchner flask
4 Winchester bottle
5 magnetic stirrer

NOTE The apparatus consists of a 2 litre Büchner flask on a magnetic stirrer, with a 2 cm diameter PVC tube inserted into it, through a rubber bung, such that the tube is just above the magnetic follower in the flask. Four holes are drilled in the tube and nipples attached by gluing into position. The pullback line and the debubble line containing the cyanogen chloride are attached to the nipples, together with the two neutralizing agents. This arrangement ensures that the cyanogen chloride has to pass down the tube and through the bulk of the flask before overflowing to waste, thus ensuring complete neutralization.

Figure 2 — On-line apparatus for destruction of cyanogen chloride

5 Reagents

All reagents shall be used according to good laboratory practice and existing national regulations. Use distilled water or water of at least equivalent purity.

5.1 **Polyoxyethylene lauryl ether** (Brij 35 solution).

Add 1 litre of water to 250 g of Brij 35. Warm and stir until dissolved.

5.2 Buffer solution A

Dissolve 2,35 g of sodium chloride (NaCl) and 7,60 g of sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$) in water. Transfer to a 1 litre volumetric flask, add 1 ml of Brij 35 solution (5.1) and dilute to volume with water. Filter the solution through a Whatman No. 1[2] (or equivalent) filter paper before use.

5.3 Buffer solution B

Dissolve 26 g of anhydrous disodium hydrogen orthophosphate (Na_2HPO_4), 10,4 g of citric acid monohydrate [$COH(COOH)(CH_2COOH)_2{\cdot}H_2O$] and 7 g of sulfanilic acid ($NH_2C_6H_4SO_3H$) in water. Transfer to a 1 litre volumetric flask, add 1 ml Brij 35 solution (5.1) and dilute to volume with water. Filter the solution through a Whatman No. 1[2] (or equivalent) filter paper before use.

5.4 **Chloramine T solution** [$CH_3C_6H_4SO_2N(Na)Cl{\cdot}3H_2O$], (*N*-chloro-4-methyl benzenesulfonamide sodium salt).

Dissolve 8,65 g of chloramine T in water. Transfer to a 500 ml volumetric flask and dilute to volume with water. Filter the solution through a Whatman No. 1[2] (or equivalent) filter paper before use.

5.5 Cyanide neutralizing solution A

Dissolve 1 g of citric acid (see 5.3) and 10 g iron(II) sulfate ($FeSO_4{\cdot}7H_2O$) in water and dilute to 1 litre.

This neutralizing solution may also be prepared by mixing 500 ml of sodium hydroxide (mass fraction of 20 %) with 70 ml sodium hypochlorite, containing a mass fraction of 5 % active chlorine.

5.6 Cyanide neutralizing solution B

Dissolve 10 g of anhydrous sodium carbonate (Na_2CO_3) in water and dilute to 1 litre.

This neutralizing solution may also be prepared by mixing 500 ml of sodium hydroxide (mass fraction of 20 %) with 70 ml of sodium hypochlorite containing a mass fraction of 5 % active chlorine.

5.7 **Potassium cyanide solution** (KCN)

WARNING — Take care: potassium cyanide is extremely toxic. See safety precautions in Clause 4.

In a fume cupboard, weigh 2 g of potassium cyanide into a 1 litre beaker. Add 500 ml of water and stir (magnetic stirrer) until all of the solid has dissolved. Store in a brown glass bottle.

5.8 **Nicotine hydrogen tartrate** [$C_{10}H_{14}N_2(C_4H_6O_6)_2{\cdot}2H_2O$].

Check the purity of the nicotine hydrogen tartrate according to ISO 13276.

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[2] Whatman No. 1 is an example of a suitable product available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this product.

5.9 Standard nicotine solutions

Check the purity of the nicotine hydrogen tartrate according to ISO 13276.

5.9.1 Stock solution

Weigh, to the nearest 0,1 mg, approximately 1,3 g of nicotine hydrogen tartrate (5.8). Dissolve in water, transfer to a 250 ml one-mark volumetric flask and dilute to the mark with water.

Nicotine stock solution may also be prepared by using nicotine or other nicotine salts of known purity.

5.9.2 Working standards

From the stock solution (5.9.1) prepare a series of at least five calibration solutions, the nicotine concentrations of which cover the range expected to be found in the test samples, e.g. 0,04 mg/ml to 0,80 mg/ml.

If 5 % acetic acid (5 times) is to be used to extract the nicotine from the test sample in 7.2, the working standards shall be made from the stock solution every 2 weeks using 5 % acetic acid instead of water.

6 Apparatus

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

6.1 Continuous flow analyser, consisting of

- sampler,
- proportioning pump,
- dialyser,
- delay coils,
- colorimeter (or equivalent) with 460 nm filter(s), and
- recorder.

See Annex B for an example of a suitable layout.

6.2 Coil for cyanogen chloride generation

A commercially available microbore mixing coil may be used for the *in-situ* generation of cyanogen chloride. Alternatively, a five-turn mixing coil may be prepared (see Annex C).

7 Procedure

7.1 Preparation of samples for analysis

Prepare the tobacco samples for analysis by grinding (the sample should totally pass through a 1 mm sieve) and determine the moisture content. If the tobacco is too wet for grinding, it may be dried at a temperature not exceeding 40 °C.

7.2 Test portion

Weigh, to the nearest 0,1 mg, approximately 250 mg, of the prepared tobacco in a 50 ml dry conical flask. Add 25 ml of water, stopper the flask and shake for 30 min.

7.3 Preparation of test extract

Filter the extract through a Whatman No. 40[3] (or equivalent) filter paper. Reject the first few millilitres of the filtrate, then collect the filtrate in an analyser cup.

If the sample is to be tested simultaneously for reducing substances (see ISO 15153) or reducing carbohydrates (see ISO 15154) or nitrates (see ISO 15517), use 25 ml of 5 % acetic acid solution (5 times) for the extraction instead of water.

Run the samples and standards through the system in the normal manner (e.g. priming with six tobacco extracts, calibration standards and samples with one intermediate calibration solution after every six samples). If sample concentrations lie outside the range of the standards, the samples shall be diluted and run again.

When using 5 % acetic acid extracts, the wash solution shall be 5 % acetic acid.

NOTE If this method is performed simultaneously with the methods described in ISO 15154 or ISO 15517, combined standards may be prepared. Combined stock solutions may precipitate after about 2 weeks.

8 Calculation

8.1 Plot a graph of peak height against equivalent nicotine concentration for all the calibration solutions.

8.2 Calculate the percentage of nicotine, w, on a dry weight basis, in the tobacco using the formula

$$w = \frac{c \times V \times 100}{m} \times \frac{100}{100 - M}$$

where

c	is the nicotine concentration, expressed in milligrams per millilitre, obtained from the calibration curve (see 8.1);
V	is the volume, in millilitres, of extract prepared (see 7.2) (normally 25 ml);
m	is the mass, in milligrams, of the sample (see 7.2);
M	the moisture content, expressed as percentage by mass, of the tobacco (see 7.1).

The test result shall be expressed to two decimal places.

9 Repeatability and reproducibility

An international collaborative study involving 12 laboratories and 3 samples conducted in 1993 showed that when single grades of tobacco were analysed by this method, the following values for repeatability limit (r) and reproducibility limit (R) were obtained.

The difference between two single results, found on different extractions by one operator using the same apparatus within a short time interval (the time it takes to analyse 40 sample cups) and without recalibration of the equipment during the time of analysis, 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 reported by two laboratories will differ by more than the reproducibility limit (R) on average not more than once in 20 cases in the normal and correct operation of the method.

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[3] Whatman No. 40 is an example of a suitable product available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this product.

Data analysis gave the estimates as summarized in Tables 1 and 2.

For the purpose of calculating r and R, one test result was defined as the yield obtained from analysing a single extract once.

Table 1 — Extraction with water

Tobacco type	**Mean content of nicotine** % (dry weight)	**Repeatability limit** r	**Reproducibility limit** R
Oriental	1,17	0,05	0,19
Flue cured	2,90	0,08	0,41
Burley	3,97	0,12	0,55

Table 2 — Extraction with 5 % acetic acid

Tobacco type	**Mean content of nicotine** % (dry weight)	**Repeatability limit** r	**Reproducibility limit** R
Oriental	1,17	0,07	0,21
Flue cured	2,90	0,11	0,67
Burley	3,97	0,13	0,97

10 Test report

When reporting results, the method used shall be specified.

Annex A
(informative)

Information about alternative analytical methods

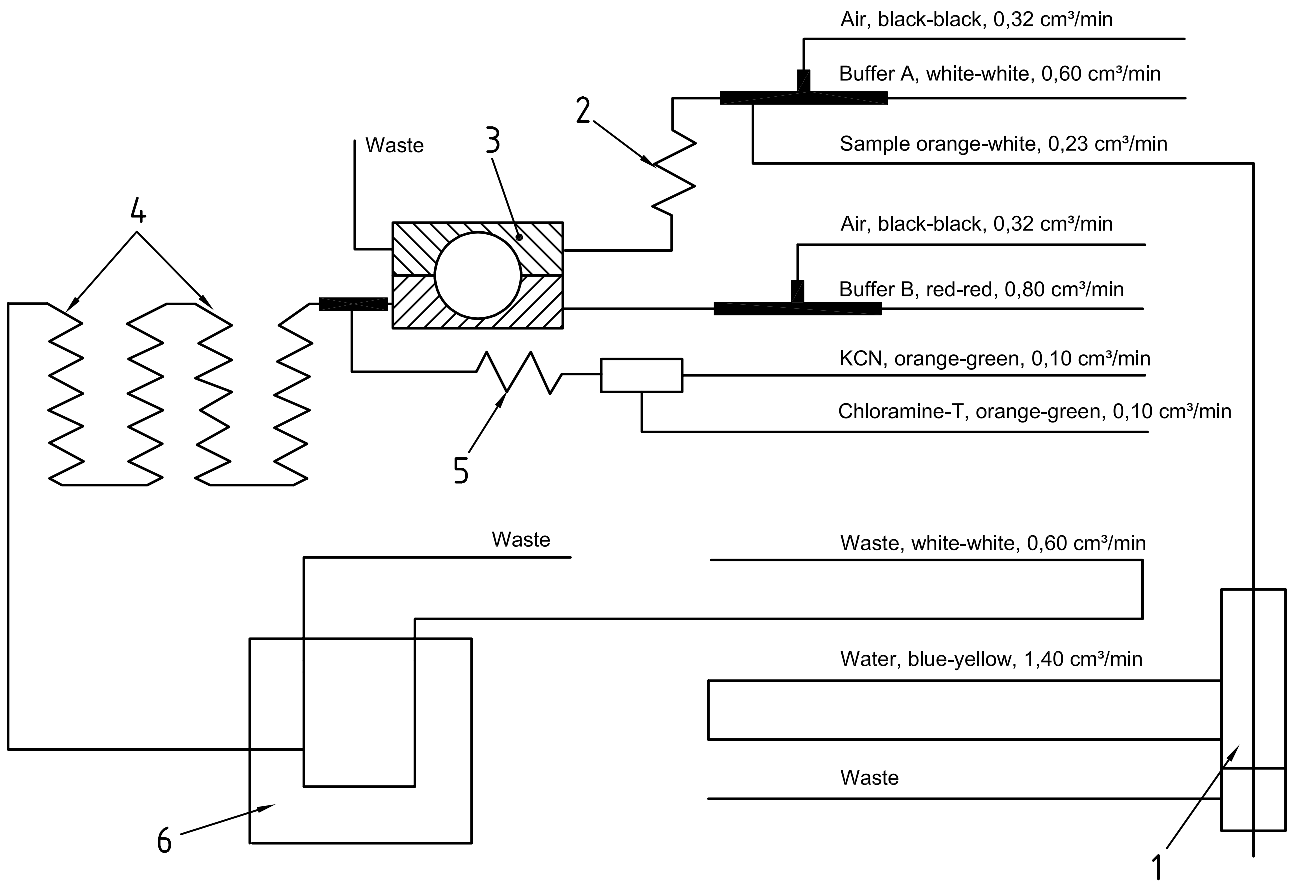
The procedure described in this International Standard uses the reaction of nicotine in the sample extract with cyanogen chloride generated from potassium cyanide and chloramine T *in-situ*.

Alternatively, it is possible to generate cyanogen chloride *in-situ* from the reaction of potassium thiocyanate and sodium hypochlorite. Several collaborative studies carried out by a CORESTA Task Force during 1989 and 1990 have shown that the alternative procedure gives equivalent results provided that the available chlorine content of the sodium hypochlorite used is between 10 % and 14 % (mass fraction).

The reaction of cyanogen bromide with aniline may also be used to determine total alkaloids. Collaborative studies by the CORESTA Task Force have shown that this reaction can also give equivalent results to the procedure used in this International Standard.

Annex B
(informative)

Example of a continuous-flow analyser



Key

1 sampler (35 s sample, 30 s wash)
2 delay coil, 10 turns (157-0226-01)[a]
3 dialyser, 12°
4 delay coils, 20 turns (157-0248-01)[a]
5 mixing coil
6 colorimeter with 460 nm filter, 2 mm × 15 mm flow cell (199-B018-02)[a] and reference cell (199-B028-01)[a]

NOTE Wash and analysis times are intended only as a guide.

[a] The part numbers given in parentheses are from Technicon. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this equipment.

Figure B.1 — Flow diagram of continuous-flow analyser

Annex C
(informative)

Preparation of a microbore five-turn mixing coil

Loop a standard orange-white (id 0,64 mm) pump tube five times around a glass tube (e.g. test tube, glass rod) with an external diameter of approximately 12 mm.

While holding the loops in place, brush them thoroughly with cyclohexanone.

Use adhesive tape to hold the loops in place while the cyclohexanone sets the tubing (about 10 h).

Remove the glass tube from the coil.

Bibliography

[1] ISO 2881, *Tobacco and tobacco products — Determination of alkaloid content — Spectrometric method*

[2] ISO 15153, *Tobacco — Determination of the content of reducing substances — Continuous-flow analysis method*

[3] ISO 15154, *Tobacco — Determination of the content of reducing carbohydrates — Continuous-flow analysis method*

[4] ISO 15517, *Tobacco — Determination of nitrate content — Continuous-flow analysis method*

ICS 65.160

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