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Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method using KSCN/DCIC

Tabac — Détermination de la teneur en alcaloïdes totaux exprimés en nicotine — Méthode par analyse en flux continu à l'aide de KSCN/DCIC

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

In 2014, the CORESTA Routine Analytical Chemistry Sub-Group (RAC) undertook a collaborative study of two methods for the determination of total alkaloids in tobacco (as nicotine) by segmented continuous-flow analysis. The two methods are ISO 15152 and a new method proposed by the China National Tobacco Quality Supervision and Test Center. In ISO 15152, cyanogen chloride is generated in situ by the reaction of potassium cyanide and chloramine T. The proposed method eliminates the use of the potassium cyanide (KCN) by employing potassium thiocyanate (KSCN) with sodium dichloroisocyanurate dihydrate (DCIC) for colour development. Each method was tested using water extracted tobacco and 5 % acetic acid extracted tobacco. Calibration standards were prepared with the same extraction solutions.

Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method using KSCN/DCIC

1 Scope

This document 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 leaf samples, stems, reconstituted tobacco sheet materials and tobacco blends.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 3696, *Water for analytical laboratory use — Specification and test methods*

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

3 Terms and definitions

No terms or definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

An aqueous extract (see the next paragraph) of the tobacco is prepared and the total alkaloids content (as nicotine) of the extract is measured by reaction of sulfanilic acid and cyanogen chloride. Cyanogen chloride is produced in situ by reaction of potassium thiocyanate (KSCN) and sodium dichloroisocyanurate (DCIC). The developed brown colour is measured at 460 nm.

A collaborative study has shown that the method gives equivalent results for water and 5 % acetic acid extracts. 5 % acetic acid extracts shall be used if total alkaloids (as nicotine) and reducing substances (see ISO 15153) or reducing carbohydrates (see ISO 15154) are to be carried out simultaneously.

5 Reagents

Use only reagents of recognized analytical grade. All reagents shall be used according to good laboratory practice.

5.1 Polyoxyethylene lauryl ether (Brij-35™[1]), a mass fraction of 30 % solution), $(C_2H_4O)_nC_{12}H_{26}O$, CAS # 9002-92-0.

1) Birj-35™ is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.2 Acetic acid, CH_3COOH, CAS # 64-19-7.

5.3 Sodium phosphate dibasic dodecahydrate, $Na_2HPO_4 \cdot 12H_2O$, CAS # 10039-32-4.

5.4 Sodium phosphate monobasic dihydrate, $NaH_2PO_4 \cdot 2H_2O$, CAS # 13472-35-0.

5.5 Sodium citrate dihydrate, $C_6H_5Na_3O_7 \cdot 2H_2O$, CAS # 6132-04-3.

5.6 Sulfanilic acid, $NH_2C_6H_4SO_3H$, CAS # 121-57-3.

5.7 Potassium thiocyanate (KSCN), CAS # 333-20-0.

5.8 Sodium dichloroisocyanurate (DCIC), $C_3Cl_2N_3NaO_3$, CAS # 51580-86-0.

5.9 Sodium carbonate, Na_2CO_3, CAS # 497-19-8.

5.10 Iron(II) sulfate heptahydrate, $FeSO_4 \cdot 7H_2O$, CAS # 7782-63-0.

5.11 Citric acid monohydrate, $C_6H_8O_7 \cdot H_2O$, CAS # 5949-29-1.

5.12 Nicotine ditartrate dihydrate, $C_{10}H_{14}N_2(C_4H_6O_6)2 \cdot 2H_2O$, CAS # 6019-06-3.

6 Preparation of solutions

6.1 General

Water used shall be high quality distilled or deionized (DI) water, free from organic contamination. The water used shall be level 1 as defined in ISO 3696.

For best results, vacuum filter all reagents through a 0,45 µm filter (see Figure 1). If necessary, vacuum filter all water used in the preparation of standards and for the sampler wash, otherwise degas the water in another way.

NOTE Millipore XX1604700 MilliSolve Kit[2], complete with 2 L flask is an example of a suitable product available commercially.

Figure 1 — Example vacuum filter set-up

2) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.2 System wash solution

Add 1 ml of polyoxyethylene lauryl ether (5.1), 30 % solution to about 800 ml water and mix. Then dilute to 1 000 ml with water. Do not store the solution longer than a week and use a clean bottle for the fresh solution.

6.3 5 % acetic acid solution

Add 50 ml of acetic acid to about 800 ml water and mix. Then dilute it to 1 000 ml with water. Do not store the solution longer than a week and use a clean bottle for the fresh solution.

6.4 Sampler wash solution

Use the extraction solution, water or 5 % acetic acid as sampler wash solution.

6.5 Potassium thiocyanate solution

Dissolve 2,88 g of potassium thiocyanate (5.7) in water. Dilute to 250 ml with water and mix well.

6.6 Sodium dichloroisocyanurate (DCIC) solution

Dissolve 2,20 g of sodium dichloroisocyanurate (5.8) and dilute to 250 ml with water. Prepare a fresh solution each day of measurement.

6.7 Neutralisation solution A

Dissolve 1 g of citric acid monohydrate (5.11) and 10 g of ferrous sulfate in about 500 ml of water.

Dilute to 1 000 ml with water and mix well.

6.8 Neutralisation solution B

Dissolve 10 g of sodium carbonate (5.9) in about 500 ml of water. Dilute to 1 000 ml with water and mix well.

6.9 Buffer solution A

Dissolve 71,6 g of sodium phosphate dibasic dodecahydrate (5.3) and 11,76 g of sodium citrate dihydrate (5.5) in about 500 ml of water. Dilute to 1 000 ml with water, add 1 ml of polyoxyethylene lauryl ether (5.1), 30 % solution mix thoroughly.

6.10 Buffer solution B

Dissolve 71,6 g of sodium phosphate dibasic dodecahydrate (5.3), 6,2 g of sodium phosphate monobasic dihydrate (5.4), 11,76 g of sodium citrate dihydrate (5.5) and 7,0 g of sulfanilic acid (5.6) in about 800 ml of water. Dilute to 1 000 ml with water, add 1 ml of polyoxyethylene lauryl ether (5.1), 30 % solution mix thoroughly.

7 Preparation of standards

7.1 General

Check the purity of the nicotine ditartrate dihydrate (5.12) according to ISO 13276. The method can also be standardized by using nicotine or other nicotine salts of known purity. In this case, an amount equivalent to the above used nicotine ditartrate dihydrate should be used.

7.2 Nicotine stock solution

Weigh 3,75 g (to the nearest 0,000 1 g) of nicotine ditartrate dihydrate (5.12) in water and dilute to 500 ml in a volumetric flask. The solution contains approximately 2,5 mg nicotine per ml, store in a refrigerator at 0 °C to 4 °C. Before use, the nicotine stock solution stored at low temperature should be equilibrated to the working temperature. Prepare a fresh solution every month.

7.3 Working standards

From the nicotine stock solution and extraction solution (water or 5 % acetic acid solution), prepare a series of at least 5 calibration solutions according to the nicotine concentration which is expected to be found in the test samples (e.g. a mass fraction of 0,5 % to 15 %). Calculate the exact concentration for each standard taking into account the purity of the nicotine ditartrate dihydrate (5.12). Store in a refrigerator at 0 °C to 4 °C. Before use, the working standards stored at low temperature should be equilibrated to the working temperature. Prepare fresh solutions every two weeks.

8 Apparatus

The laboratory needs the usual laboratory apparatus and, in particular, the following items.

8.1 Continuous-flow analyser, consisting of

— autosampler;

— peristaltic pump;

— chemistry manifold with dialyser and delay coils;

— photometric detector equipped with a 460 nm filter;

— data acquisition system or recorder.

See Figures A.1 and A.2 in Annex A for examples of suitable flow diagrams.

9 Procedure

9.1 Preparation of samples for analysis

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

9.2 Test portion

Weigh to the nearest 0,1 mg, approximately 250 mg, of the ground tobacco into a 50 ml conical flask. Add 25 ml of the extraction solution (water or 5 % acetic acid solution). Stopper and shake for about 30 min at about 150 r/min.

9.3 Preparation of test extract

Filter the extract through a quantitative filter paper such as Whatman No 40[3] (or equivalent ashless, quantitative filter paper) filter paper, rejecting the first few ml of the filtrate, then collect the filtrate. After filtration, immediately run the sample extraction solution and working standards through the

3) Whatman No 40 is an example of an available product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

continuous-flow analyser in the normal manner. If sample concentration lies outside the range of the standards, the sample shall be diluted and run again.

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

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

10 Calculation

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

10.2 Calculate the percentage of nicotine, *w*, on a dry weight basis, in the tobacco using the Formula (1)

$$w = \frac{c \times V \times 100}{m} \times \frac{100}{100 - M} \quad (1)$$

where

c is the nicotine concentration, expressed in milligrams per millilitre, obtained from the calibration curve (7.3);

V is the volume, in millilitres, of the sample (see 9.2), normally 25 ml;

m is the mass, in milligrams, of the sample (see 9.2);

M the moisture content, expressed as percentage by mass, of the tobacco (see 9.1).

The test result shall be expressed to two decimal places.

11 Repeatability and reproducibility

In 2014, an interlaboratory study involving 19 laboratories and eight samples (four straight grade tobaccos, a fire-cured cigarette, a blended cigarette, CM7 Monitor Test Piece, and the Kentucky Reference Product 3R4F) was conducted. The repeatability limit (*r*) and reproducibility limit (*R*) were calculated for this new KSCN/DCIC method and ISO 15152 using both water and 5 % acetic acid extractions (see Tables 1 and 2).

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 approximately 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 no more than the reproducibility limit (R) on average not more than once in 20 cases in the normal and correct operation of the method.

Table 1 — Extraction with water

Tobacco type	Mean content of nicotine (% dry weight)		Repeatability *r*		*r*CV[a]		Reproducibility *R*		*R*CV[b]	
	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC
Fire-cured	1,76	1,81	0,05	0,04	2,87	2,37	0,12	0,27	6,67	14,81
Burley	4,77	4,77	0,11	0,11	2,35	2,23	0,61	0,52	12,76	10,97
Oriental	0,98	0,98	0,04	0,03	4,07	3,51	0,24	0,19	24,10	19,21
Dark Sun-cured	3,71	3,71	0,08	0,06	2,19	1,73	0,48	0,45	12,89	12,02
Fire-cured cigarette	2,11	2,07	0,06	0,04	2,71	2,15	0,34	0,30	15,90	14,33
Blended cigarette	2,02	2,07	0,03	0,03	1,59	1,51	0,2	0,29	9,65	13,86
CM7	2,2	2,22	0,05	0,05	2,41	2,29	0,28	0,30	12,48	13,43
3R4F	2,15	2,12	0,06	0,06	2,90	2,61	0,29	0,24	13,70	11,52

[a] *r*CV is *r*/mean × 100 %.

[b] *R*CV is *R*/mean × 100 %.

Table 2 — Extraction with 5 % acetic acid

Tobacco type	Mean content of nicotine (% dry weight)		Repeatability *r*		*r*CV[a]		Reproducibility *R*		*R*CV[b]	
	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC	ISO 15152	KSCN/ DCIC
Fire-cured	1,74	1,76	0,04	0,04	2,39	2,28	0,13	0,19	7,51	11,04
Burley	4,53	4,53	0,11	0,08	2,39	1,87	0,41	0,56	8,99	12,35
Oriental	0,96	1,00	0,04	0,03	4,04	3,38	0,14	0,14	14,66	13,7
Dark Sun-cured	3,51	3,60	0,07	0,06	2,06	1,60	0,26	0,44	7,46	12,29
Fire-cured cigarette	2,04	2,05	0,05	0,04	2,57	1,79	0,18	0,27	8,69	13,19
Blended cigarette	1,98	2,02	0,04	0,03	2,14	1,59	0,18	0,23	8,99	11,23
CM7	2,11	2,17	0,05	0,04	2,33	1,95	0,17	0,27	8,19	12,25
3R4F	2,08	2,10	0,06	0,05	2,80	2,34	0,26	0,25	12,55	11,52

[a] rCV is r/mean × 100 %.

[b] RCV is R/mean × 100 %.

12 Test report

The test report shall provide the total alkaloids (as nicotine) results to precision of two decimal places. It shall also provide all details necessary for the identification of the sample.