INTERNATIONAL STANDARD

ISO 22980

First edition

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

PROOF/ÉPREUVE

Reference number
ISO 22980:2020(E)

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Published in Switzerland

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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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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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.

Collaborative studies have 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-35TM 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, $Na_2HPO_4 \cdot 12H2O$, CAS # 10039-32-4.

5.4 Sodium phosphate monobasic dihydrate, $NaH_2PO_4 \cdot 2H2O$, CAS # 13472-35-0.

5.5 Sodium citrate dihydrate, $C_6H_5Na_3O_7 \cdot 2H2O$, 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, $C_6H_8O_7 \cdot H2O$, 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.