INTERNATIONAL STANDARD

ISO 13276

Third edition
2020-01

Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid

Tabac et produits du tabac — Détermination de la pureté de la nicotine — Méthode gravimétrique à l'acide tungstosilicique

Reference number
ISO 13276:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of 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 www.iso.org/iso/foreword.html.

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

This third edition cancels and replaces the second edition (ISO 13276:2017), which has been technically revised. The main changes compared to the previous edition are as follows:

- the Warning notice has been updated;
- the error in Formula (1) in Clause 8 has been corrected.

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.

Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of any other restrictions prior to use.

1 Scope

This document specifies a method for the gravimetric determination of the purity of nicotine using tungstosilicic acid.

The method is applicable to pure nicotine or nicotine salts used to calibrate analytical methods for the determination of nicotine in the field of tobacco, tobacco products and smoke analysis.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and 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

Complex formation of nicotine or its salts with tungstosilicic acid to form insoluble nicotine silicotungstate. Determination of the precipitate mass by filtration using either a sintered glass crucible in combination with oven-drying or an ashless filter paper in combination with incineration.

5 Reagents

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

5.1 **Tungstosilicic acid solution** (CAS of tungstosilicic acid: 12027-43-9).

Dissolve 12 g of dodeca-tungstosilicic acid ($H_4[Si(W_3O_{10})4] \cdot xH_2O$) in 100 ml of water.

Avoid the use of the other forms of tungstosilicic acid such as $4H_2O{\cdot}SiO_2 10WO_3{\cdot}3H_2O$ or $4H_2O{\cdot}SiO_2 12WO_3{\cdot}20H_2O$ as they do not yield crystalline precipitates with nicotine. Tungstosilicic acid of the CAS 11130-20-4 and 12027-38-2 may be used for this method provided sufficiently crystalline precipitation occurs.

5.2 Hydrochloric acid solution, HCl, a volume fraction of 20 %.

Dilute 20 ml of hydrochloric acid, ρ_{20} (HCl) = 1,18 g/ml, to 100 ml with water.

5.3 Hydrochloric acid solution, HCl, a volume fraction of 0,1 %.

Dilute 5 ml of hydrochloric acid solution (5.2) to 1 l with water.

5.4 Nicotine solution, $\rho(C_{10}H_{14}N_2)$ = 0,1 mg/ml.

Dissolve 2,5 mg of nicotine ($C_{10}H_{14}N_2$) in water using a volumetric flask (6.1) and dilute to 25 ml with water.

6 Apparatus

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

6.1 Volumetric flask, of 25 ml capacity.

6.2 Beakers, of 250 ml capacity.

6.3 Watch glasses.

6.4 Glass stirring rods.

6.5 Desiccator, containing an effective drying agent.

6.6 Apparatus for glass filter filtration procedure.

6.6.1 Sintered glass crucible (Gooch type), of porosity 2 (40 µm to 100 µm).

6.6.2 Filter flask (Buchner flask).

6.6.3 Vacuum source.

6.6.4 Laboratory oven, capable of maintaining a temperature of (120 ± 5) °C.

6.7 Apparatus for filter paper filtration procedure.

6.7.1 Ashless filter paper.[1]

6.7.2 Porcelain or platinum crucibles.

6.7.3 Gas or **electric Bunsen burner,** capable of maintaining a temperature higher than 600 °C.

6.7.4 Furnace, capable of maintaining a temperature higher than 600 °C (optional).

6.8 Analytical balance, with a resolution of 0,1 mg.

---

[1] The ashless filter paper Whatman No. 42 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.

7 Procedure

7.1 Precipitation procedure

A suitable amount of replicates should be measured. Weigh, to the nearest 0,000 1 g, approximately 0,1 g of the nicotine alkaloid (or the equivalent amount of nicotine salt) (m) in each of, for example, five 250 ml beakers (6.2) equipped with glass stirring rods (6.4).

Add 100 ml of water to each beaker. Add 2 ml of 20 % hydrochloric acid solution (5.2) to each beaker and stir. Do not remove the stirring rod.

Add slowly 15 ml of the tungstosilicic acid solution (5.1) while stirring constantly during the addition. Cover each beaker with a watch glass (6.3), leaving the stirring rod in place, and allow to stand overnight. Before filtering, stir the precipitate to ensure that it settles quickly and is of a crystalline form. Check for complete precipitation with a few extra drops of the tungstosilicic acid solution.

7.2 Filtration procedure

7.2.1 General

Filtration can be performed by either of the procedures given in 7.2.2 or 7.2.3.

7.2.2 Glass filter filtration procedure

Dry each glass filter crucible (6.6.1) in the oven (6.6.4) at (120 ± 5) °C to constant mass (±1 mg). Store in the desiccator (6.5).

Weigh, to the nearest 0,000 1 g, each glass crucible (6.6.1) (m_1) and filter the precipitate directly into the glass filter using the Buchner flask (6.6.2) and vacuum source (6.6.3). Ensure that the precipitate is removed from the sides of the beaker and the glass stirring rod by washing into the filter with hydrochloric acid solution (5.3) approximately three times using 15 ml each. Discard the washings.

Rinse with a further aliquot portion of hydrochloric acid solution (5.3) (up to 400 ml may be required) which should be collected and tested with a few drops of nicotine solution (5.4) to ensure that no opalescence occurs, i.e. all tungstosilicic acid has been removed.

Dry each glass crucible and precipitate in the oven (6.6.4) for 3 h at (120 ± 5) °C. Allow to cool in the desiccator and weigh to the nearest 0,000 1 g (m_2). Place the filters back in the oven for 1 h, allow to cool and reweigh. Repeat, if necessary, until a constant mass (±1 mg) is obtained.

7.2.3 Filter paper filtration procedure

Filter the precipitate directly onto an ashless filter paper (6.7.1). Ensure that the precipitate is removed from the sides of the beaker and the stirring rod by washing into the filter with hydrochloric acid solution (5.3) approximately three times using 15 ml each. Discard the washings.

Rinse with a further aliquot portion of hydrochloric acid solution (5.3) (up to 400 ml may be required) which should be collected and tested with a few drops of nicotine solution (5.4) to ensure that no opalescence occurs, i.e. all tungstosilicic acid has been removed.

Dry each crucible (6.7.2) on the Bunsen burner (6.7.3) or in the furnace (6.7.4) at 600 °C until constant mass (±1 mg). Store in the desiccator (6.5).

Weigh, to the nearest 0,000 1 g, each crucible (6.7.2) (m_1). Transfer the filter paper with the precipitate to the crucible. Place the crucible on a silica triangle resting on a tripod, heat gently at first and then ignite with the Bunsen burner (6.7.3). The crucible contents have to be broken up very carefully to ensure complete removal of the carbon. The final residue should be greenish/yellow in colour. Allow to cool in the desiccator (6.5) and weigh to the nearest 0,000 1 g (m_2). Repeat the heating process until a constant mass (±1 mg) is obtained.

NOTE After ignition of the filter paper, it might be convenient to leave the crucible in the furnace (6.7.4) at above 600 °C overnight. This technique ensures that no further heating is required.

8 Expression of results

The nicotine purity or the nicotine salt purity, *NP*, expressed as a percentage by mass, is given by Formula (1):

$$NP = \frac{(m_2 - m_1) \times C}{m} \times 100 \qquad (1)$$

where

m_1 is the mass, in milligrams, of the dried empty crucible;

m_2 is the mass, in milligrams, of the crucible with precipitate after drying (7.2.2) or ignition (7.2.3);

C is a factor depending on the filtration procedure:

~0,101 2 for the glass filter filtration procedure (7.2.2);

~0,114 1 for the filter paper filtration procedure (7.2.3);

m is the nicotine equivalent mass, in milligrams, of the sample.

When the method is used to determine the purity of a nicotine salt, calculate the nicotine equivalent mass m from the mass of nicotine salt m_s by using Formula (2):

$$m = m_s \times \frac{162,2}{M_s} \qquad (2)$$

where M_s is the molecular mass of the nicotine salt.

Take the arithmetic mean of the five determinations as the test result. Report the result to one decimal place.

Should nicotine salts be contaminated with residues of unreacted nicotine, results above 100 % can be obtained by using this method as the method is specific for nicotine. These anomalous results can be considered as qualified for calibration purposes.

9 Repeatability and reproducibility

Two interlaboratory studies have been conducted using this method; one in 1993 using both pure and degraded nicotine and one in 2014 using a nicotine salt (glass filter filtration, 7.2.2) from which the values for the repeatability limit (r) and the reproducibility limit (R) given in Table 1 were obtained.

The difference between two test results found on different analyses by one operator using the same apparatus within a short time interval 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 test 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.

Data analysis gave the estimates summarized in Table 1.

Table 1 — Estimates given by data analysis

Nicotine type	Year	Number of laboratories	Number of replicates	Mean purity of nicotine %	Repeatability limit *r*	Reproducibility limit *R*
Pure (>99 %)	1993	17	1	98,8	2,2	3,8
Degraded	1993	17	1	96,7	1,6	3,2
Tartrate (99,7 ± 0,3)	2014	12	5	99,3	0,7	1,0

For the purpose of calculating *r* and *R* in the 1993 study, one test result was defined as the yield obtained from analysing one sample once.

NOTE 1 Estimation of *R* in the 1993 study based on average of five single determinations gave the following values: 2,9 for degraded nicotine and 3,3 for pure nicotine.

NOTE 2 If nicotine salts are contaminated with residues of unreacted nicotine, results above 100 % can be obtained by using this method as the method is specific for nicotine. These anomalous results can be considered as qualified for calibration purposes.

10 Test report

The test report shall contain the following information:

a) the method used;

b) the result obtained;

c) all details required for the complete identification of the sample;

d) all operating conditions not specified in this document, or regarded as optional, as well as any circumstances that may have affected the result.