INTERNATIONAL STANDARD

ISO 11454

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Tobacco and tobacco products — Determination of vapour-phase nicotine in air — Gas-chromatographic method

Tabac et produits du tabac — Dosage de la nicotine en phase gazeuse dans l'air — Méthode par chromatographie en phase gazeuse

Reference number
ISO 11454:1997(E)

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.

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.

International Standard ISO 11454 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products.*

Annex A of this International Standard is for information only.

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INTRODUCTION

The method described in this International Standard has been recommended after studies by members of CORESTA (Co-operation Centre for Scientific Research Relative to Tobacco) as being suitable for the quantitative determination of vapour-phase nicotine in ambient air.

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Tobacco and tobacco products — Determination of vapour-phase nicotine in air — Gas-chromatographic method

1 SCOPE

This International Standard specifies a method for the determination of vapour-phase nicotine in ambient air. If the method is used to determine nicotine derived from tobacco smoke, it has to be accepted that whilst airborne nicotine is a qualitative marker to confirm that smoking has taken place, on the basis of research, its amount cannot be quantitatively extrapolated to other smoke components.

NOTE 1 For comments concerning limitations of use and field of application, see annex A, reference [1].

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 edition indicated was 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 edition of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 648:1977, Laboratory glassware - One-mark pipettes.

ISO 1042:1983, Laboratory glassware - One-mark volumetric flasks.

ISO 13276:1997, Tobacco and tobacco products - Determination of nicotine purity - Gravimetric method using tungstosilicic acid.

3 DEFINITIONS

For the purposes of this International Standard, the following definitions apply.

3.1 vapour-phase nicotine: That nicotine which is not bound to aerosol particulate matter.

3.2 ambient air: The air contained in specified indoor or outdoor environments.

4 PRINCIPLE

Drawing a known volume of air at approximately 1 l/min through an adsorbent sampling tube containing the specified resin. Transfer of the resin to a glass vial and addition of an internal standard. Desorption of nicotine and internal standard into a solvent. Determination by gas chromatography using a thermionic detector.

5 REAGENTS

All reagents shall be of recognized analytical reagent grade.

All volumetric flasks and pipettes shall comply with class A of ISO 1042 and class A of ISO 648 respectively.

5.1 Ethyl acetate, chromatographic grade.

5.2 Triethylamine, minimum purity 99 %, for modifying ethyl acetate solvent.

5.3 Nicotine, minimum purity 99 %.

Until use, store at 0 °C to 4 °C in the dark.

The content should be determined in accordance with ISO 13276.

5.4 Quinoline, mimimum purity 99 % for use as internal standard.

5.5 Adsorbent resin, comprising an agglomerated resin [1]) consisting of styrene crosslinked to divinyl benzene and having the form of microspheroids with a surface area of approximately 725 m^2/g.

5.6 Extraction solvent, for dissolving nicotine adsorbed on adsorbent resin (5.5).

Prepare a solution of 0,01 % (V/V) of triethylamine (5.2) in ethyl acetate (5.1).

5.7 Nicotine standard solutions

5.7.1 Primary standard solution

Weigh accurately about 100 mg of nicotine (5.3). Transfer to a 100 ml standard volumetric flask and dilute to the mark with extraction solvent (5.6).

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[1]) XAD-4 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.

5.7.2 Secondary standard solution

Pipette 1 ml of the primary standard solution (5.7.1) into a 100 ml standard volumetric flask and dilute to volume with the extraction solvent (5.6).

5.8 Quinoline standard solutions

5.8.1 Primary standard solution

Weigh accurately about 100 mg of quinoline (5.4). Transfer to a 100 ml standard volumetric flask and dilute to the mark with the extraction solvent (5.6).

5.8.2 Secondary standard solution

Pipette 10,0 ml of the primary standard solution (5.8.1) into a 100 ml standard volumetric flask and dilute to volume with the extraction solvent (5.6).

5.9 Calibration solutions

Prepare at least two sets, each of five calibration standard solutions, covering the range of concentrations to be expected in the test sample, by adding aliquot portions of the nicotine secondary standard solution (5.7.2), for example 10 µl, 20 µl, 50 µl, 100 µl and 200 µl (resulting in 0,1 µg to 2,0 µg nicotine per vial), to five 2 ml glass vials (6.4). Add 50 µl of the quinoline secondary standard solution (5.8.2) and 1 ml of the extraction solvent (5.6) to each vial. Cap and shake to mix.

> NOTE 2 Neither the exact volume in the vial nor the replication of volume between duplicates of the same nicotine concentration is important provided that the ratio of nicotine to quinoline in the test solution is maintained.

5.10 Gases: Helium, hydrogen and compressed air for operation of the gas chromatograph.

6 APPARATUS

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

6.1 Sampling pump, calibrated for an air flow rate of 1 l/min.

6.2 Glass sorbent sampling tubes, with both ends flame-sealed, 7 cm x 6 mm od (outside diameter) and 4 mm id (inside diameter), containing two sections of 20/40 mesh adsorbent resin separated by glass wool spacers. [2)]

6.3 Gas chromatograph, with temperature programming capability, thermionic detector (see note 3), digital electronic integrator, and (optional) autosampler.

[2)] Catalogue No. 226-93 from SKC Inc., 863 Valley View Road, Eighty Four, 15330 Pennsylvania, USA. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Recommended capillary columns are:

a) 30 m x 0,32 mm id fused silica capillary, coated with 1,0 µm film of 5 % phenyl methylsilicone (preferred); or

b) 30 m x 0,53 mm id fused silica capillary, coated with 1,5 µm film of 5 % phenyl methylsilicone.

These columns will require an on-column or split/splitless capillary inlet. However, a packed column inlet could be used with column b).

NOTE 3 Thermionic detectors are a specific class of gas chromatography detectors which exhibit enhanced selectivity/sensitivity to certain classes of chemicals based on selective surface-enhanced ionization. They are also known as "alkali flame ionization" and "nitrogen-phosphorus" detectors.

6.4 Sample containers, consisting of 2 ml borosilicate glass vials with polytetrafluorethylene (PTFE)-lined septum closures (e.g., autosampler vials).

6.5 Analytical balance, accurate to 0,001 g.

6.6 One-mark pipettes, of capacity 1 ml and 10 ml.

6.7 Standard volumetric flasks, of capacity 100 ml.

6.8 Micropipettes, capable of delivering 5 µl, 10 µl, 20 µl and 50 µl, or microdispenser, capable of delivering volumes from 5 µl to 200 µl.

6.9 Mechanical shaker, having characteristics that ensure that a desorption efficiency value (see 7.5) close to 100 % can be achieved.

7 PROCEDURE

7.1 Sample collection

Break off both ends of the sorbent resin tube (6.2) ensuring that diameter of the opening is at least half of the internal diameter of the tube. Select the resin at one end of the tube to be for primary collection of the sampled air and connect the other end to the sampling pump (6.1) with tubing. Set the pump rate to a value between 0,5 l/min and 1,25 l/min and collect the air to be sampled for the period required. Record the values of the flow rate set and the sampling time in order that V (8.2) may be calculated.

NOTE 4 A minimum sampling time of 1 h is recommended.

If the measured concentration requires subsequently to be adjusted to standard temperature and pressure (see 8.3), record the average temperature and barometric pressure during the sampling period.

After sampling, cap the sorbent tubes with plastics caps unless the samples are to be tested immediately. Store either at room temperature in the dark for a maximum of 2 weeks or in a freezer; the latter is recommended. If stored in a freezer, samples are stable for at least 4 weeks.

7.2 Sample preparation and test portion

7.2.1 Test samples

For each sample, transfer the glass wool plug and the adsorbent resin from the primary end of the sampling tube (6.2) to a 2 ml glass vial (6.4). Transfer the adsorbent resin from the backup end and its bracketing glass wool plugs to a second vial. To each vial add 50 µl of the quinoline secondary standard solution (5.8.2) and 1 ml of the extraction solvent (5.6). Cap and shake for 30 min.

7.2.2 Blank

Concurrently with the samples, take three unopened sampling tubes. Open both ends and transfer the glass wool plugs and resin to separate vials and add the secondary internal standard and the extraction solvent and treat as described in 7.2.1.

7.3 Gas chromatography

Set up and operate the gas chromatograph, integrator, and autosampler (optional) according to manufacturer's instructions. Suitable operating conditions for the gas chromatograph are as follows:

Helium carrier gas flow: approximately 2 ml/min or 83 kPa for column a) (6.3), or approximately 15 ml/min or 103 kPa for column b) (6.3)
Initial oven temperature (time): 150 °C (0 min)
Temperature programme rate: 5 °C/min to 180 °C
Injector temperature: 250 °C
Injection volume: 1 µl to 2 µl
Injection mode: a) split (ratio approximately 10 : 1); or b) direct or on-column
Detector temperature: 300 °C
Detector sensitivity: sufficient to give a signal/noise ratio greater than 50 for nicotine in 0,1 µg/ml calibration standard solution.

The total analysis time is approximately 6 min.

NOTES

5 The pressure is given as a guide for setting the flow by measurement of head pressure.

6 For use of splitless injection, see annex A, reference [6].

7.4 Determination

Chromatograph one set of calibration standard solutions and samples, and a second set of calibration solutions, and obtain integrated peak areas for quinoline and nicotine. Calculate the nicotine-to-quinoline peak area ratio for each. Calculate mean results for all calibration solutions analysed at a given concentration and construct a calibration curve using peak area ratios as ordinate and the nicotine content in micrograms as abscissa values. Fit the calibration data to either a linear or a second-order least-squares regression model (whichever is more appropriate for the instrument used: expected $R^2 >$ 0,990). Calculate the mass of nicotine in each sample from the nicotine-to-quinoline peak area ratio.

7.5 Determination of desorption efficiency

For each of 20 unused sorbent sampling tubes, open the tube at both ends and transfer the glass wool plug from the tube inlet and the adsorbent resin from the primary end to a 2 ml glass vial (6.4). Spike the resin with nicotine by adding to each of five vials 10 µl aliquot portions of the nicotine secondary standard solution (5.7.2), to a further five vials add 20 µl and to five vials add 50 µl. The remaining five vials are used as blanks. Cap and store all vials in the same manner as the test samples.

Add 50 µl of the quinoline secondary standard solution (5.8.2) to all vials and 1 ml of extraction solvent (5.6) and analyse the spiked samples and blanks for nicotine as for the determination of the test samples in 7.4.

The desorption efficiency DE for nicotine for each level of nicotine in the spiked samples is given by the following equation:

$$\mathrm{DE} = \frac{A_s - B_s}{C_s}$$

where

A_s is the mean mass of nicotine, in micrograms, found in the spiked sample vials;

B_s is the mean mass of nicotine, in micrograms, found in the blank vials;

C_s is the mean mass of nicotine, in micrograms, added to spike the sample.

Use an analysis of variance or t-test to determine whether the value of DE is different for the three levels of spiking used ($P < 0,05$). If the value of DE is different, construct a plot of DE values against the mass of nicotine added. Use this plot to obtain the value of DE to be used in the calculation of the mass of nicotine in the air sample (see 8.1). If the value is equivalent for the three levels but different from 100 % when tested by a t-test using the pooled results, take as the DE value the pooled arithmetic mean.

Repeat the whole determination at least once until consistent values are obtained (t-test, P not less than 0,05).

8 EXPRESSION OF RESULTS

8.1 Calculation of the total mass of nicotine in the sample

The total mass of nicotine, W, in the sample, expressed in micrograms, is given by the equation:

$$W = \frac{(A_p - B_p) + (A_b - B_b)}{\mathrm{DE}}$$

where

A_p is the mass of nicotine, in micrograms, found in primary section of the sorbent resin sample tube;

B_p is the mean mass of nicotine, in micrograms, found in the primary sections of all blank tubes (described in 7.2);

A_b is the mass of nicotine, in micrograms, found in the backup section of the sample tube;

B_b is the mean mass of nicotine, in micrograms, found in the backup section of all blank tubes (described in 7.2);

DE is the desorption efficiency determined in accordance with 7.5.

If the quantity of nicotine collected on the backup section of the sample tube exceeds 5 % of the total, then breakthrough has occurred and the entire sample should be considered invalid. In such instances, sampling should be repeated using lower flow rates, shorter collection times, or both, so as not to exceed the capacity of the resin adsorbent tube.

8.2 Calculation of the nicotine content in the air

The nicotine content, N, in the sampled air, expressed in micrograms per cubic metre, is given by the equation:

$$N = \frac{W \times 1000}{V}$$

where

W is the total mass of nicotine in the sample, in micrograms;

V is the volume of air sampled, in litres;

1000 is the conversion factor from litres to cubic metres.

8.3 Calculation of the nicotine content in the air in relation to standard conditions of temperature and pressure

If desired or if necessary because of pump characteristics, the nicotine content found in sampled air adjusted to standard conditions of temperature and pressure, N_{stand} expressed in micrograms per cubic metre, is given by the equation:

$$N_{\text{stand}} = N \times \frac{1013}{p} \times \frac{(T+273)}{298}$$

where

N is the nicotine content in accordance with 8.2;

p is the barometric pressure of air sampled, in millibars;

T is the temperature of air sampled, in degrees Celsius;

1013 is the rounded standard pressure, in millibars;

298 is the rounded standard temperature, in kelvins.

9 PRECISION

An international collaborative study involving 7 laboratories and 6 samples conducted in 1991 (see reference [5]) gave the following values for repeatability limit (r) and reproducibility limit (R):

Table 1

Mean concentration of nicotine, µg/m^3	Repeatability limit r	Reproducibility limit R
4,0	0,5	1,3
13,2	2,4	2,8
34,7	4,0	5,8

10 POTENTIAL INTERFERENCE

Although an atmosphere containing tobacco smoke contains quinoline at approximately 1 % of the nicotine concentration, the use of quinoline as internal standard (IS) in this method is appropriate under most circumstances. In field-sampling situations, the volume of air sampled in conjunction with typical nicotine concentrations results in 0 µg to 2 µg nicotine collected on the sorbent resin. For sample concentrations in this range, the mass of quinoline also collected on the resin is below detection limits and poses no interference with the 5 µg quinoline added as IS.

If this method is used in situations resulting in large quantities of collected nicotine (> 10 µg), then the standard method should be modified. Recommended modifications are:

1) decrease sampling flow rate and/or duration;

2) extract sorbent resin in a larger solvent volume and add the specified aliquot of IS solution to only 1 ml of extract;

3) use an increased amount of quinoline as IS;

4) use *N*-ethylnornicotine as IS (see reference [3]).