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Environmental tobacco smoke — Determination of vapour phase nicotine and 3-ethenylpyridine in air — Gas-chromatographic method

Fumée de tabac ambiante — Dosage de la nicotine et de la 3-éthénylpyridine en phase vapeur dans l'air — Méthode par chromatographie en phase gazeuse

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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.

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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.

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ISO 18145 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Introduction

Nicotine and 3-ethenylpyridine (3-EP) are commonly used tracers for environmental tobacco smoke (ETS). Nicotine and 3-EP are highly selective for tobacco smoke and both have been used as markers of ETS in indoor air. Among the attributes of an ideal ETS tracer is the need to be unique or highly specific to tobacco smoke in sufficient concentrations in air to be measured easily at realistic smoking rates, and in constant proportion to the other components of ETS for a variety of tobacco blends and environmental conditions (see [1]). While nicotine is the more commonly used marker, it is not an ideal marker for several reasons, most notable of which are its adsorptive tendencies and unpredictable decay rate. A measure of the nicotine concentrations may underestimate ETS during smoke generation, due to the ability of nicotine to be adsorbed on building materials and room furnishings, therefore being depleted from the ETS at a rate faster than most other components. On the other hand, an overestimation of ETS may result from the slow desorption of nicotine over time. Nicotine concentration measurements are a strong indication that smoking has occurred. However, nicotine concentrations do not necessarily indicate the presence or concentration of any other ETS components. In contrast, 3-EP has been shown to track the vapour phase of ETS as measured by CO and FID (flame ionization detector) response exactly (see [2]). Due to this correlation, 3-EP may be a better tracer for ETS (see [3], [4], [5], [6], [7]).

High concentrations of ETS have become a concern for potential health effects due to the annoyance and irritation experienced by individuals. Therefore, a need to establish reliable estimation methods of ETS levels is a priority. Although not related to ETS, a workplace threshold limit value (TLV) for nicotine has been set by the National Institute for Occupational Safety and Health (NIOSH) in the United States at 0,5 mg/m^3. For various indoor environments, observed nicotine concentrations can range from not detected (ND) to about 70 μg/m^3, with values usually at the lower end of this range (see [8], [9]). Due to the low concentrations typically found for nicotine, more sophisticated analytical procedures and equipment are often required for quantification in indoor air. Other methods have also been reported for the determination of nicotine in indoor air (see [10], [11], [12], [13], [14]).

Approximately 95 % of ETS nicotine is found in the vapour phase of the aerosol and it can be efficiently collected by air sampling using sorbent tubes. Early studies indicate that not all of freshly generated ETS particulate phase is trapped on sorbent resin (see [11], [15]). The trapping of particulate matter by sorbent beds has been suggested by another report to be nearly quantitative (see [16]). 3-Ethenylpyridine concentrations in real-world environments are usually one-third that of nicotine and are found exclusively in the vapour phase (see [10], [17]). This method has been used in a variety of real-world ETS studies (see [9], [18], [19]).

Environmental tobacco smoke — Determination of vapour phase nicotine and 3-ethenylpyridine in air — Gas-chromatographic method

1 Scope

This International Standard specifies a method for the sampling and determination of nicotine and 3-ethenylpyridine (3-EP) in environmental tobacco smoke (ETS). This method is applicable to personal and area sampling.

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 648:1977, *Laboratory glassware — One-mark pipettes*

ISO 1042:1998, *Laboratory glassware — One-mark volumetric flasks*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
environmental tobacco smoke
ETS
mixture of aged and diluted exhaled mainstream smoke and aged and diluted sidestream smoke

3.2
nitrogen-phosphorus detector
NPD
selective and highly sensitive detection device used for nitrogen- and phosphorus-containing organic compounds

4 Principle

The test method is based on the collection of nicotine and 3-EP by adsorption on a sorbent resin, extraction of nicotine and 3-EP from the sorbent resin, and determination by gas chromatography (GC) with nitrogen selective detection (see [3]). A sorbent sampling tube, through which a known volume of air is drawn, is used to adsorb nicotine and 3-EP in indoor air. Upon completion of the sampling, the contents of the tube are transferred to a 2 ml autosampler vial. Desorption is obtained by an ethyl acetate solution containing 0,01 % triethylamine and a specified quinoline (the internal standard) concentration. A GC-NPD is injected with an aliquot of the desorbed sample. Area ratios are acquired from the injection of standards and are compared with the areas of the resulting nicotine and 3-EP peaks, which have been divided by the area of the internal standard peak.

5 Limits and detection

The method specified in this International Standard allows the estimation with the following lower limits of nicotine and 3-EP concentration. At a sampling rate of 1,0 l/min, the limits of detection (LOD) and quantification (LOQ) are as follows:

a) for nicotine

— 0,17 µg/m^3 (LOD) and 0,56 µg/m^3 (LOQ) for a 1 h sampling period, and

— 0,02 µg/m^3 (LOD) and 0,07 µg/m^3 (LOQ) for an 8 h sampling period;

b) for 3-EP

— 0,08 µg/m^3 (LOD) and 0,28 µg/m^3 (LOQ) for a 1 h sampling period, and

— 0,01 µg/m^3 (LOD) and 0,03 µg/m^3 (LOQ) for an 8 h sampling period.

Both LOD and LOQ can be reduced by increasing the sensitivity of the thermionic-specific detector.

6 Reagents

All reagents shall be of a recognized analytical grade.

6.1 Sorbent, macroreticular polystyrene-divinylbenzene copolymer beads, 420 nm to 841 nm (20/40 mesh), 725 m^2/g mean surface area.[1)]

6.2 Compressed air, for detector gas (< 0,1 ppm hydrocarbon).

6.3 Ethyl acetate, chromatographic quality.

6.4 4-Ethenylpyridine (4-EP), 95 %, commercially available isomer of 3-ethenylpyridine.

6.5 Compressed helium, for carrier or detector makeup gas, or both, 99,995 % grade.

6.6 Compressed hydrogen, for detector gas, 99,995 % grade.

6.7 Nicotine, 99 %.

6.8 Quinoline (internal standard), 99 %.

6.9 Triethylamine, 99 %.

6.10 Modified ethyl acetate solvents

6.10.1 Modified ethyl acetate solvent with internal standard

Add 0,5 ml of triethylamine and 30 µl of quinoline (approximately 8 µg/ml) to a freshly opened 4 l bottle of ethyl acetate. Shake or stir to mix. The solvent is modified with a volume fraction of 0,01 % triethylamine to prevent any adsorption of nicotine on the glass walls of the vials (see [20]).

Store in a refrigerator (at about 4 °C) when not in use. Prepare fresh solvent at least every 12 months.

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1) XAD-4 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

6.10.2 Modified ethyl acetate solvent without internal standard

Add 0,5 ml of triethylamine to a freshly opened 4 l bottle of ethyl acetate. Shake or stir to mix.

6.11 Nicotine and 4-EP standard solutions

In order to keep the amount of internal standard constant for both standards and samples, the same batch of modified solvent that is used to prepare standard solutions shall be used to extract samples. Therefore, whenever a new batch of modified solvent is prepared, a new batch of standard solutions shall be prepared. Otherwise, if standards and samples contain different amounts of internal standard, the exact amounts in both solutions must be known precisely, and the regression and equations in 10.2 must be modified to reflect the different internal standard concentrations.

6.11.1 Primary standard of nicotine

Prepare a primary standard of nicotine (400 µg/ml) by weighing 100 mg of nicotine directly into a 250 ml volumetric flask. Dilute to the mark with solvent (6.10.1) and shake to mix.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standards at least every 6 months.

6.11.2 Primary standard of 4-EP

Prepare a primary standard of 4-EP (500 µg/ml) by weighing 100 mg of 4-EP into a 200 ml volumetric flask Dilute to the mark with solvent (6.10.1) and shake to mix.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standard at least every 6 months.

6.11.3 Secondary standard of nicotine and 4-EP

Prepare a secondary standard of nicotine (4,8 µg/ml) and 4-EP (2 µg/ml) by transferring 3,0 ml of primary nicotine standard and 1,0 ml of primary 4-EP standard to a 250 ml volumetric flask. Dilute to the mark with solvent (6.10.1) and shake to mix.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standard at least every 6 months.

6.11.4 Working standards of nicotine and 4-EP

Prepare five working standards covering the expected concentration range of the samples by transferring defined volumes of the secondary standard (6.11.3) to 100 ml volumetric flasks. Dilute to the mark with solvent (6.10.1) and shake to mix. Recommended volumes are 100,0 ml, 30,0 ml, 15,0 ml, 6,0 ml and 2,0 ml, which correspond to concentrations of 6,0 µg, 1,80 µg, 0,90 µg, 0,36 µg and 0,12 µg/1,25 ml for nicotine, and 2,5 µg, 0,75 µg, 0,375 µg, 0,15 µg and 0,05 µg/1,25 ml for 4-EP.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standards at least every 6 months.

6.12 Spiking standards of nicotine and 4-EP (without internal standard)

6.12.1 Primary spiking standard of nicotine

Prepare a primary spiking standard of nicotine (400 µg/ml) by weighing 100 mg of nicotine directly into a 250 ml volumetric flask. Dilute to the mark with solvent (6.10.2) and shake to mix.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standard at least every 6 months.

6.12.2 Primary spiking standard of 4-EP

Prepare a primary spiking standard of 4-EP (500 µg/ml) by weighing 100 mg of 4-EP directly into a 200 ml volumetric flask. Dilute to the mark with solvent (6.10.2) and shake to mix.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standard at least every 6 months.

6.12.3 Secondary spiking standard of nicotine and 4-EP

Prepare a secondary spiking standard of nicotine (9,6 µg/ml) and 4-EP (4,0 µg/ml) by transferring 6,0 ml and 2,0 ml of the primary nicotine and 4-EP spiking standards, respectively, to a 250 ml volumetric flask. Dilute with solvent (6.10.2) and shake to mix.

Store in low-actinic borosilicate glass screw-cap jars in a freezer (at –10 °C or less) when not in use. Prepare fresh standard at least every 6 months.

7 Apparatus

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

7.1 Sample collection system

7.1.1 Bubble flowmeter or **mass flowmeter**, for sample pump calibration.

7.1.2 Plastic caps, for capping sorbent tubes after sampling.

7.1.3 Personal sampling pump, portable constant-flow sampling pump, calibrated for the desired flow rate (up to 1,5 l/min).

7.1.4 Tube breaker, to break sealed ends from sorbent tubes.

7.1.5 Tube holder, with clip attachment for attaching tube to clothing or objects.

7.1.6 Sorbent tube, glass tube with both ends flame-sealed, approximately 7 cm length with 6 mm outside diameter and 4 mm inside diameter, containing one section of 120 mg sorbent resin[2)]. The resin is held in place inside the glass tube by a plug of glass wool (outlet end) and a plug of glass wool and metal lockspring (inlet end).

7.2 Analytical system

7.2.1 Gas chromatograph, with a nitrogen-phosphorus (thermionic-specific) detector (NPD) and autosampler (optional).

7.2.2 GC column, fused silica capillary column, 30 m in length with 0,32 mm inside diameter, coated with a 1,0 µm film of 5 % phenyl methylpolysiloxane.

7.2.3 Chromatography data acquisition system, for measuring peak areas electronically.

7.2.4 Sample containers, borosilicate glass autosampler vials, of 2 ml capacity, with PTFE-lined septum closures.

7.3 Dispensing pipettes, of 1,25 ml capacity.

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2) Catalog No. 226-170, supplied by SKC, Inc., Eighty Four, 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.

7.4 Triangular file, for scoring and breaking open sample tubes.

7.5 Forceps, for assisting transfer of sorbent tube contents from tube to autosampler vial.

7.6 Glass wool removal tool, for assisting transfer of sorbent tube contents from tube to autosampler vial.

7.7 Wrist-action shaking device, for solvent extraction.

7.8 One-mark pipettes, complying with class A of ISO 648:1977.

7.9 One-mark volumetric flasks, complying with class A of ISO 1042:1998.

8 Sampling procedure

8.1 Calibration of the personal sampling pump

Adjust the potentiometer on the air sampling pump to the specified flow rate ($\leqslant$ 1,5 l/min).

Calibrate the personal sampling pump prior to and immediately following sampling. For calibration, connect the flowmeter to the inlet of the sorbent tube. Measure the flow with the prepared sorbent tube in place between the pump and the flowmeter.

If using a mass flowmeter, record the volumetric flow rate (q_V) of the air sampling pump. If using a bubble flowmeter, generate several soap-film bubbles in the flowmeter, and allow them to wet the surface before recording any actual measurements. Measure with a stopwatch the time for a soap-film bubble to travel a known volume. Obtain five replicate measurements and compute the mean time.

Calculate the volumetric flow rate of the pump, q_V, expressed in litres per minute, from the following equation:

$$q_V = \frac{V_s}{t_s} \qquad (1)$$

where

V_s is the volume measured with flowmeter, expressed in litres (l);

t_s is the average time, expressed in minutes (min), for the soap-film bubble to travel V_s in the bubble flowmeter.

8.2 Sorbent tube and personal sampling pump preparation

Prepare the sorbent tubes by breaking both ends with a tube breaker tool (to an opening of at least 2 mm diameter or one-half of the tube inside diameter, whichever is the larger). Place the sorbent tube into the tubing, or in a holder, connected to the pump with the inlet end exposed to the atmosphere. Adjust the pump potentiometer to the flow rate required ($\leqslant$ 1,5 l/min). Measure and record the flow rate (l/min) using a flowmeter.

Prepare and treat a minimum of two sorbent tubes in the same manner as the sample tubes (break, measure flows, cap and transport). Label and process these tubes as flow blanks.

8.3 Sample collection

Turn on the pump and record the start time for sampling.

Collect samples at the calibrated flow rate for a specified time period, generally a minimum of 1 h.

Upon completion of the sampling time, turn off the pump and record the stop time.