INTERNATIONAL STANDARD

ISO 18145

First edition
2003-07-15

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

Reference number
ISO 18145:2003(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

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.

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.

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.

---

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:

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

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.

Re-measure and record the flow rates. Use the average flow rate ($\bar{q}_V$) for calculations.

Calculate the average flow rate, $\bar{q}_V$, from the following equation:

$$\bar{q}_V = \frac{\bar{q}_{V\mathrm{i}} + \bar{q}_{V\mathrm{f}}}{2} \quad (2)$$

where

$\bar{q}_{V\mathrm{i}}$ is the initial flow rate;

$\bar{q}_{V\mathrm{f}}$ is the final flow rate.

Remove the sorbent tube from the pump and cap the sorbent tube.

Analyse or store (at $\leqslant 0$ °C) the sorbent tubes immediately. Samples remain stable for at least 8 weeks in storage.

9 Analytical procedure

9.1 General

If the samples were refrigerated, allow them to equilibrate to room temperature prior to analysis.

9.2 Sorbent resin desorption and extraction

The extraction process is carried out in a nicotine-free environment. In addition, immediately prior to analysis the analyst shall cleanse their hands with soap and water and refrain from smoking or coming into contact with nicotine-containing surfaces or environments. This procedure shall be followed from the beginning of the extraction process through to the loading of the autosampler tray.

The extraction is performed using the modified ethyl acetate solvent (6.10.1).

Transfer the sorbent tube contents to an autosampler vial for extraction. Remove the sorbent tube caps and enlarge the openings, by use of a file, for easier transfer. Remove the lockspring and glass wool and transfer them together with the sorbent resin contents to the vials by use of the removal tool and forceps. Label the vial and add 1,25 ml of solvent with internal standard (6.10.1) to the vial. Then seal the vials and place them in the holding tray. Upon completing the transfer of all tube contents, agitate the holding tray for 30 min to aid desorption by use of the wrist-action shaking device.

Prepare two new (not previously opened) sorbent tubes as blanks. Label and process these tubes as laboratory blanks. If the tubes have been refrigerated, they shall be allowed to equilibrate to room temperature for at least 1 h.

One of two methods may be used to extract any remaining resin beads from within the tubes: either the glass wool may be used to push the beads out, or a stream of air may be used to flush them out.

9.3 Loading autosampler

At the beginning of the autosampler queue, load one set of the calibration standards. Then, load the samples and blanks. Finally, load the second set of calibration standards.

If more than 40 samples are to be analysed, prepare an extra set of standards. Load the samples after the first set of standards, then load half of the samples followed by a second set of standards, then load the other half of the samples and finally the third set of standards. Prepare the necessary number of standards to allow no more than 40 samples to be analysed between standards.

Load the wash [ethyl acetate with 0,01 % triethylamine and without internal standard (6.10.2)] and waste vials on the autosampler.

9.4 Gas chromatographic (GC) determination of nicotine and 3-ethenylpyridine

9.4.1 Setting up the GC apparatus

Set up the apparatus and operate the gas chromatography system in accordance with the manufacturer's instructions. Perform analyses using a GC fitted with a nitrogen-phosphorus detector and an autosampler equipped for split/splitless injection. The autosampler typically uses default settings for the injection sequence, and 1 µl or 2 µl of sample are injected with a 30 s splitless period.

The following operating conditions have been found to be suitable.

a) Temperatures

- injector: 225 °C
- oven initial temperature: 50 °C
- hold time: 1 min
- programme step 1
  - rate: 10 °C/min
  - final temperature: 215 °C
  - hold time: 0 min
- programme step 2
  - rate: 20 °C/min
  - final temperature: 275 °C
  - hold time: 2 min
- detector: 300 °C

b) Gas flows

- He as carrier: 4 ml/min (103 kPa)
- H_2 as detector: 3 ml/min
- air as detector: 75 ml/min
- He as makeup: 15 ml/min

Detector gas flow rates are illustrative. Detector manufacturer operating instructions and guidelines should be followed.

NOTE Approximate retention times are

- 3-EP, 4-EP: 8,5 min,
- quinoline: 13,5 min, and
- nicotine: 15 min.

9.4.2 Analysis of samples and blanks

Make a trial injection of the first calibration standard to verify the GC operation for correct peak location, resolution, and shape and sensitivity of the detector.

Using the data acquisition system, obtain integrated areas and peak ratios of analyte-to-quinoline for the standards, samples and blanks. Compare the area ratios and calculate the nicotine and 3-EP sample concentrations using the calibration curves.

NOTE Response factors for 3-EP and 4-EP have been determined to be equivalent (see [21]) and the two isomers have the same retention time and peak shape under the listed chromatographic conditions.

9.4.3 Constructing the calibration curves

9.4.3.1 Nicotine calibration curve

Plot the mean peak area ratio of nicotine-to-quinoline on the y-axis versus the nicotine concentration (in micrograms per 1,25 ml) on the x-axis. Fit the data into a second-order polynomial regression model with $1/x$ weighting.

Other regression models may be deemed more appropriate and, if so, may be used instead of the second-order weighted regression. If other models are used, the appropriate regression equations shall be substituted in the calculation in 10.2.

9.4.3.2 4-EP calibration curve

Plot the mean peak area ratio of 4-EP-to-quinoline on the y-axis versus 4-EP concentration (in micrograms per 1,25 ml) on the x-axis. Fit the data into a second-order polynomial regression model with $1/x$ weighting.

Other regression models may be deemed more appropriate and, if so, may be used instead of the second-order weighted regression. If other models are used, the appropriate regression equations shall be substituted in the calculation in 10.2.

9.4.4 Determination of desorption efficiency

9.4.4.1 General

For every different lot of sorbent tubes, determine the desorption efficiency. The desorption efficiency is the decimal fraction (or percentage) of nicotine and 4-EP recovered in the desorption process.

9.4.4.2 Preparation of spiked vials for determination of desorption efficiency

Prepare 20 sorbent tubes by breaking both ends with a tube breaker tool. Transfer all the contents of the tubes into 2 ml autosampler vials. Prepare three sets of five spiked vials. To the first set, add 10 µl of the secondary spiking standard (0,096 µg nicotine; 0,04 µg 4-EP) directly to the resin bed, being careful to coat the bed and not the glass walls. Add to the next two sets, 20 µl (0,192 µg nicotine; 0,08 µg 4-EP) and 50 µl (0,48 µg nicotine; 0,20 µg 4-EP), respectively. The remaining set of five vials shall be used as blanks.

Cap and store (at $\leqslant$ 0 °C) all vials. Choose the storage time as the average time required to analyse field samples, since the desorption efficiency may depend on the length of time stored.

It is important that the standard solutions (6.11) which contain quinoline (the internal standard) are not used to spike the vials for determination of desorption efficiency.

10 Expression of results

10.1 Calculation of desorption efficiency

The desorption efficiency, ω_{DE}, expressed as mass fraction in percent (%), is defined by the equation:

$$\omega_{DE} = \frac{m_{AR}}{m_{AS}} \times 100\ \% \tag{3}$$

where

m_{AR} is the average analyte recovery mass, in micrograms (µg);

m_{AS} is the mass of the analyte spiked onto the sorbent resin, in micrograms (µg).

The desorption efficiency may be dependent on the amount of analyte collected on the sorbent resin. If so, construct a plot of the desorption efficiency versus mass of analyte found experimentally (not the amount used to spike).

The desorption efficiency is usually 100 % over the calibration ranges suggested in 6.11 (see [10] and [20]).

If the desorption efficiency is less than 100 %, then read the desorption efficiency from the curves generated (or, if no curves were generated, use the simple arithmetic means).

10.2 Calculation of analyte concentration in the sample

10.2.1 The mass of the analyte in the sample, m_A, expressed in micrograms (µg), is given by the equation:

$$m_A = m_S - m_B \tag{4}$$

where

m_S is the mass of the analyte determined in the sample tube, in micrograms (µg);

m_B is the average mass of the analyte in the blank tubes, in micrograms (µg).

Either the laboratory blanks (9.2) or the flow blanks (8.2) may be used, whichever are deemed more appropriate. In general, it is expected that flow blanks (8.2) will be the more appropriate.

10.2.2 The analyte concentration is related to the peak area ratio for analyte-to-quinoline using the following regression model.

Second-order polynomial regression model:

$$y = A + Bx + Cx^2 \tag{5}$$

where

y is the peak area ratio (analyte:quinoline);

x is the analyte concentration.

10.2.3 The corrected analyte mass, m_{AC}, in micrograms (µg), is obtained from the following equation:

$$m_{AC} = \frac{m_A}{\omega_{DE}} \tag{6}$$

where

m_A is the total analyte mass, in micrograms (µg), calculated from Equation (4);

ω_{DE} is the desorption efficiency, expressed as a mass fraction in percent (%), calculated from Equation (3).

NOTE For calculations involving desorption efficiency, the decimal fraction (e.g. 1,00) is used instead of the percentage (e.g. 100 %).

10.3 Calculation of analyte content in the air

The analyte content, ρ_{AA}, in the sampled air, in micrograms per cubic metre (µg/m^3), is given by the equation:

$$\rho_{AA} = \frac{m_{AC} \times 1\,000}{t \times \bar{q}_V} \qquad (7)$$

where

m_{AC} is the mass of analyte, in micrograms, calculated from Equation (6);

1 000 is the conversion factor for the conversion of litres to cubic metres, in the sampled air, in litres per cubic metre (l/m^3);

t is the time elapsed during sample collection (see 8.3), in minutes (min), obtained from pump start and stop times;

$\bar{q}_V$ is the average of the initial and final flow rates of the sampling pump, in litres per minute (l/min), as found in 8.1 and 8.3.

11 Laboratory performance criteria and quality assurance

Guidance concerning performance criteria and a summary of quality assurance measures that should be achieved within each laboratory are provided in Annex A.

12 Repeatability and reproducibility

The precision data were determined from an experiment organized and analysed in accordance with ISO 5725 [22] in 1998 involving 11 laboratories for nicotine and 3-ethenylpyridine, and 6 levels. Data from 2 laboratories for nicotine and 3 laboratories for 3-EP contained outliers. The outliers were not included in the calculation of the repeatability standard deviations and the reproducibility standard deviations. Precision data were determined to vary linearly with mean level over the range 0,40 µg to 2,01 µg per sample for nicotine and 0,25 µg to 0,90 µg per sample for 3-EP. These relationships are the following:

— repeatability standard deviation, s_r = a $\times$ m

— reproducibility standard deviation, s_R = A $\times$ m

where m is the mean sample level, in micrograms per sample.

The values of a and A are listed in Table 1.

Table 1 — Values a and A

Analyte	a	A
Nicotine	0,075	0,126
3-Ethenylpyridine	0,052	0,119

13 Test report

The test report shall give the ambient nicotine and 3-ethenylpyridine concentrations, in micrograms per cubic metre, and shall include all conditions which may affect the result (e.g. atmosphere, sampling time and sampling rate). It shall also give all details necessary for the identification of the atmosphere under test.