CORESTA RECOMMENDED METHOD N° 50

ENVIRONMENTAL TOBACCO SMOKE - DETERMINATION OF NICOTINE AND 3-ETHENYLPYRIDINE IN THE VAPOUR PHASE

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1. FIELD OF APPLICATION

Due to the annoyance and irritation experienced by individuals from high concentrations of environmental tobacco smoke (ETS), a concern arises for its potential health effects. This concern brings forth a need to establish reliable methods for estimating ETS levels. 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) at 0.5 mg/m^3. Observed nicotine concentrations, in various indoor environments, can range from not detected (ND) to about 70 $\mu g/m^3$, with values usually at the lower end of this range (see Ref. 3.1, 3.2). 3-Ethenylpyridine (3-EP) concentrations are typically 10-50% of the observed nicotine levels. Sophisticated analytical procedures and equipment are often required for quantification in indoor air due to these typically low concentrations found for nicotine and 3-EP. Other methods have also been reported for the determination of 3-EP and/or nicotine in indoor air (see Ref. 3.3, 3.4, 3.5, 3.6, 3.7).

The method described here is for the sampling (personal and area sampling) and determination of nicotine and 3-EP in ETS. The collection of nicotine and 3-EP by adsorption on a sorbent resin, extraction from the sorbent resin, and determination by gas chromatography (GC) with nitrogen selective detection are the bases of this test method (see Ref. 3.8).

Nicotine and 3-EP are commonly used tracers for ETS. Both are highly selective for tobacco smoke and have been used as markers of ETS in indoor air. An ideal ETS tracer needs 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 Ref. 3.9, 3.10). While nicotine is the more commonly used marker, it's adsorptive tendencies and unpredictable decay rate make it a less than ideal marker. During smoke generation, nicotine concentrations may underestimate ETS due to its ability to adsorb on building materials and room furnishings, therefore being depleted from 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. While measurements of nicotine concentration are a strong indication that smoking has occurred, they do not necessarily indicate the presence or concentration of any other ETS components. In contrast, 3-EP has been shown to track exactly the vapor-phase of ETS as measured by CO and FID (see Ref. 3.11). Due to this correlation, 3-EP may be a better tracer for the vapor phase of ETS (see Ref. 3.8, 3.10, 3.12, 3.13, 3.14).

Air sampling by use of sorbent tubes can efficiently collect ETS nicotine found in the vapor-phase of the aerosol (vapor phase nicotine is approximately 95% of total ETS nicotine). While some early studies indicate that not all freshly generated ETS particulate phase is trapped on XAD-4 resin, another report suggests the trapping of particulate matter by sorbent beds to be nearly quantitative (see Ref. 3.4, 3.15, 3.16). 3-Ethenylpyridine is found exclusively in the vapor-phase (see Ref. 3.3, 3.17). This method has been used in a variety of real-world ETS studies (see Ref. 3.2, 3.18, 3.19).

2. DEFINITIONS

2.1. *Environmental Tobacco Smoke (ETS)*
A mixture of aged and diluted exhaled mainstream smoke and aged and diluted sidestream smoke.

2.2. *XAD-4 resin*
Macroreticular polystyrene-divinylbenzene copolymer beads.

2.3. *Nitrogen-phosphorus detector (NPD)*
A selective and highly sensitive detection device used for nitrogen- and phosphorus-containing organic compounds.

3. REFERENCES

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4. PRINCIPLE

A known volume of air is drawn through an XAD-4 sorbent sampling tube to adsorb the nicotine and 3-EP present in the air stream. The contents of the tube are transferred to a 2 ml autosampler vial upon the completion of the sampling. Desorption is accomplished 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. APPARATUS

5.1. Bubble flowmeter or mass flowmeter, for sample pump calibration.

5.2. Personal sampling pump, portable constant-flow sampling pump, calibrated for the desired flow rate (up to 1.5 l/min).

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

5.4. XAD-4 Sorbent tube (with plastic caps), glass tube with both ends flame-sealed, approximately 7 cm long with 6 mm outside diameter and 4 mm inside diameter, containing one section of 120 mg, 20/40 mesh XAD-4 resin. 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). (Catalog No. 226-170, SKC Inc., Eighty Four, Pennsylvania, USA).

5.5. Gas chromatograph, with a nitrogen-phosphorus (thermionic-specific) detector (NPD) and autosampler (optional). Recommended GC column is 30 m by 0.32 mm inside diameter fused silica capillary column, coated with a 1.0 µm film of 5% phenyl methylpolysiloxane. Chromatography data acquisition system, for measuring peak areas electronically.

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

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

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

5.9. Wrist-action shaking device, for solvent extraction.

5.10. Necessary general laboratory equipment (tube holder, dispensing pipets, forceps, one mark pipets, one mark volumetric flasks, etc.) for the preparation of samples, standards and reagents.

6. REAGENTS

6.1. Ethyl acetate (chromatographic quality) for desorbing nicotine from XAD-4 resin and preparing standard solutions.

6.2. 4-Ethenylpyridine (4-EP), 95%, commercially available isomer of 3-ethenylpyridine.

6.3. Nicotine (99+%) for preparation of standard solutions.

6.4. Quinoline (99+%) for use as internal standard.

6.5. Triethylamine (99+%) for modifying ethyl acetate solvent.

6.6. Gases: Compressed air (<0.1 ppm hydrocarbon), compressed helium (99.995% grade) and compressed hydrogen (99.995% grade) for gas chromatograph operation.

7. STANDARDS

Modify ethyl acetate solvent by adding 0.5 ml triethylamine and 30 µl quinoline (resulting in approximately 0.01% v/v triethylamine concentration and 8 µg/ml quinoline concentration) to a freshly opened 4 l bottle of ethyl acetate and shaking or stirring to mix. The solvent is modified with triethylamine to prevent any adsorption of nicotine on the glass walls of the vials (see Ref. 3.20). Use modified solvent (referred to hereafter as solvent with internal standard) to prepare necessary standards and sample solutions.

Modify ethyl acetate solvent by adding 0.5 ml triethylamine to a freshly opened 4 l bottle of ethyl acetate and shaking or stirring to mix. Use modified solvent (referred to hereafter as solvent without internal standard) to prepare necessary spiking standards and spiking sample solutions and for rinsing glassware, etc.

7.1. Nicotine and 4-EP standard solutions: (1) *Primary standard of nicotine (400 µg/ml)* - weigh 100 mg of nicotine directly into a 250 ml volumetric flask, dilute to the mark with solvent with internal standard, and shake to mix. (2) *Primary standard of 4-EP (500 µg/ml)* - weigh 100 mg of 4-EP into a 200 ml volumetric flask, dilute to mark with solvent with internal standard, and shake to mix. (3) *Secondary standard of nicotine and 4-EP* - transfer 3.0 ml of primary nicotine standard and 1.0 ml of primary 4-EP standard to a 250 ml volumetric flask, dilute to mark with solvent with internal standard, and shake to mix.

7.2. Spiking standard solutions: (1) *Primary spiking standard of nicotine (400 µg/ml)* - weigh 100 mg of nicotine directly into a 250 ml volumetric flask, dilute to mark with solvent without internal standard, and shake to mix. (2) *Primary spiking standard of 4-EP (500 µg/ml)* - weigh 100 mg of 4-EP directly into a 200 ml volumetric flask, dilute to mark with solvent without internal solvent, and shake to mix. (3) *Secondary spiking standard of nicotine (9.6 µg/ml) and 4-EP (4.0 µg/ml)* - transfer 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 without internal standard, and shake to mix.

7.3. Prepare five calibration standard solutions *that cover the expected concentration range of interest* by transferring defined volumes of the secondary standard of nicotine and 4-EP to 100 ml volumetric flasks, diluting to the mark with solvent with internal standard, and shaking to mix. Recommended volumes are 100.0, 30.0, 15.0, 6.0, and 2.0 ml which correspond to concentrations of 6.0, 1.80, 0.90, 0.36, and 0.12 µg/1.25 ml for nicotine and 2.5, 0.75, 0.375, 0.15, and 0.05 µg/1.25 ml for 4-EP, respectively.

8. PROCEDURES

8.1. *Air Pumping System Calibration*

Adjust the potentiometer on the air sampling pump to the specified flow rate (≤ 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 XAD-4 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 the time for a soap-film bubble to travel a known volume with a stopwatch. Obtain five replicate measurements, and compute the mean time.

Calculate the volumetric flow rate, q_V, expressed in liters per minute (l/min), from the following equation:

$$q_V = \frac{V_S}{t_S} \quad (1)$$

where

V_S is the volume measured with flowmeter, expressed in liters (l)

t_S the average time for soap-film bubble to travel a known volume in the bubble flowmeter, expressed in minutes (min).

8.2. *Sample Collection*

Break off both ends of the XAD-4 tubes with a tube breaker tool to an opening of at least 2 mm diameter or one half of the tube inside diameter, whichever is larger. Prepare two extra XAD-4 tubes in the same manner, label and process these tubes as flow blanks. Position the sorbent tube into 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 (≤ 1.5 /min). Measure and record the flow rate (l/min) through the use of a flowmeter. 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:

$$\bar{q}_v = \frac{\bar{q}_{v_i} + \bar{q}_{v_f}}{2} \quad (2)$$

where

$\bar{q}_{v_i}$ is the initial flow rate;

$\bar{q}_{v_f}$ is the final flow rate.

Remove sorbent tube from pump and cap sorbent tube.

8.3. *Sample Preparation*

Carry out the extraction process in a nicotine-free environment. Immediately prior to analysis, cleanse hands with soap and water and refrain from smoking or coming into contact with nicotine-containing surfaces or environments. Follow this procedure from the beginning of the extraction process through to the loading of the autosampler tray. Perform the extraction using the modified ethyl acetate solvent with internal standard. Transfer the contents of the sorbent tube to an autosampler vial for extraction. Remove the sorbent tube caps and enlarge the openings by using a file. Remove the lockspring and glass wool and transfer the sorbent resin contents to the vials by using the removal tool and forceps. Label the vial and add 1.25 ml of solvent with internal standard to the vial. Seal the vials and place in the holding tray. Using the wrist-action shaking device, agitate the holding tray for 30 min to aid desorption. Prepare and analyze two previously unopened XAD-4 tubes (blanks) concurrently with samples.

8.4. *Gas Chromatography*

Load one set of the calibration standards at the beginning of the autosampler queue followed by the samples and blanks. Finally, load the second set of calibration standards. (If more than 40 samples are being analyzed, prepare an extra set of standards. Load the samples after the first set of standards, then half of the samples, a second set of standards, the other half of the samples and finally the third set of standards. Prepare the needed number of standards to allow no more than 40 samples to be analyzed between standards.) Load wash (ethyl acetate with 0.01% triethylamine and without internal standard) and waste vials on the autosampler. Set up the apparatus and operate the gas chromatography system in accordance with the manufacturer's instructions. Analyze 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 is injected with a 30 sec splitless period. Operating conditions for the GC are as follows:

Injector: 225 °C
Oven: Initial temperature 50 °C, Hold time 1 min
Program Step 1: Rate 10 °C/min, Final temperature 215 °C, Hold time 0 min
Program Step 2: Rate 20 °C/min, Final temperature 295 °C, Hold time 1 min
Detector: 300 °C
He carrier gas flow: 4 ml/min (15 psig)
H_2 detector gas flow: 3 ml/min
Air detector gas flow: 75 ml/min
He makeup gas flow: 15 ml/min
3-EP, 4-EP retention time: 8.5 min
Quinoline retention time: 13.5 min
Nicotine retention time: 15 min

8.5. *Determination*

Obtain integrated peak areas and calculate peak area ratios of analyte to quinoline for the standards, samples, and blanks by use of a data acquisition system. Compare the area ratios and calculate the nicotine and 3-EP sample amounts, using the calibration curves.

Note: Response factors for 3-EP and 4-EP have been determined to be equivalent (see Ref. 3.21) and the two isomers have the same retention time and peak shape under the listed chromatographic conditions.

Plot the mean peak area ratio of analyte to quinoline on the y-axis versus analyte concentration (µg/1.25 ml) on the x-axis. Fit the data into an appropriate regression model; a second-order polynomial regression model with 1/x weighting is suggested.

8.6. *Determination of Desorption Efficiency*

Determine the desorption efficiency (the decimal fraction or percentage of nicotine and 4-EP recovered) for every different lot of sorbent tubes. Break both ends of 20 XAD-4 tubes with a tube breaker tool. Transfer all the tube contents into 2 ml autosampler vials. Prepare three sets of 5 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). To the next two sets add 20 µl (0.192 µg nicotine; 0.08 µg 4-EP) and 50 µl (0.48 µg nicotine; 0.20 µg 4-EP), respectively. Use the remaining set of 5 vials as blanks. Cap and store all vials. Choose the storage time and temperature as average times which field samples experience as desorption efficiency may be dependent on storage conditions

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

$$\omega_{DE} = \frac{m_{AR}}{m_{AS}} \times 100 \qquad (3)$$

where

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

m_{AS} is the mass of the analyte spiked onto the XAD-4 resin, expressed in micrograms (µg).

The desorption efficiency may be dependent on the amount of analyte collected on the XAD-4 resin. If so, construct a plot of desorption efficiency versus weight of analyte found experimentally (not the amount used to spike). The desorption efficiency is usually 100 % over the calibration ranges suggested in previously (see Ref. 3.10, 3.20). 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).

8.7. *Calculations*

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

$$m_A = m_S - m_B \qquad (4)$$

where

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

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

Note: Either the laboratory blanks (Sec. 8.2) or the flow blanks (Sec. 8.1) may be used whichever are deemed more appropriate. In general, it is expected that flow blanks (Sec. 8.1) will be the more appropriate.

The analyte concentration is related to the peak area ratio for analyte to quinoline.
Calculate the analyte concentration by use of the following second-order regression model (or other appropriate model):

$$y = A + Bx + Cx^2 \qquad (5)$$

where

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

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

$$m_{AC} = \frac{m_A}{\omega_{DE}} \qquad (6)$$

where

m_A is the total analyte mass, expressed in micrograms (µg), calculated by equation (4);

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

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

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

$$\rho_{AA} = \frac{m_{AC} \times 1000}{t \times \bar{q}_V} \quad (7)$$

where

m_{AC} is the mass of analyte, expressed in (µg), calculated by equation (6);

1000 is the conversion factor for the conversion of liters to cubic meters, in the sampled air, expressed in liters per cubic meter (l/m^3);

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

$\bar{q}_v$ is the average of initial and final flow rates of the sampling pump, expressed in liters per minute (l/min).

9. POTENTIAL INTERFERENCE

Bias in detection can occur if ETS nicotine is collected on the resin at >10 µg due to the presence of quinoline (internal standard) in ETS at low levels (about 1% of the nicotine level). Alternative procedures for employing the internal standard method of quantitation are recommended if large quantities of ETS (i.e., sufficient to yield more than 10 µg nicotine) are to be sampled. Alternative methods are as follows:

The use of larger volumes of extraction solvent with internal standard to dilute the sample quantitatively lowers the ETS quinoline and nicotine concentrations. This alternative method will keep the concentration of internal standard quinoline constant within the solvent. The nicotine concentration in the most concentrated sample should be less than or equal to the quinoline concentration in the solvent, to prevent significant interference.

An alternative method from the one described here would be to use N'-ethylnornicotine as the internal standard (see Ref. 3.21).

10. REPEATABILITY AND REPRODUCIBILITY

The precision data were determined from an experiment organized and analyzed in accordance with ISO 5725 guidelines in 1998 involving 11 laboratories for nicotine and 3-ethenylpyridine, and 6 levels (see Ref. 3.22). 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/sample for nicotine and 0.25 µg to 0.90 µg/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, expressed in µg/sample.

The values a and A are listed in the following table:

Table 1 – Values a and A

Analyte	a	A
Nicotine	0.075	0.126
3-Ethenylpyridine	0.052	0.119

11. LIMITS AND DETECTION

The method specified allows the estimation within the following limits of nicotine and 3-EP content. At a sampling rate of 1.0 l/min, limits of detection (LOD) and quantification (LOQ) are, respectively, 0.17 µg/m^3 and 0.56 µg/m^3 for a 1 h sampling period and 0.02 µg/m^3 and 0.07 µg/m^3 for an 8 h sampling period for nicotine. LOD and LOQ for 3-EP, under the same conditions, are, respectively, 0.08 µg/m^3 and 0.28 µg/m^3 for a 1 h sampling period and 0.01 µg/m^3 and 0.03 µg/m^3 for an 8 h sampling period. Both LOD and LOQ can be reduced by increasing the sensitivity of the thermionic-specific detector (See Ref. 3.22).