CORESTA RECOMMENDED METHOD N° 52

ENVIRONMENTAL TOBACCO SMOKE – ESTIMATION OF ITS CONTRIBUTION TO RESPIRABLE SUSPENDED PARTICLES – METHOD BASED ON SOLANESOL DETERMINATION

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1. FIELD OF APPLICATION

Environmental tobacco smoke (ETS) is an aerosol that contains both vapor and particulate phase components. However, due to their nature, the two aerosol phases rarely correlate well. An accurate determination of ETS levels in indoor air, therefore, requires a proper tracer of both phases. One critical aspect of an ideal ETS tracer is that it should "remain in a fairly consistent ratio to the individual contaminant of interest or category of contaminants of interest (for example, suspended particulates) under a range of environmental conditions…" (see Ref. 3.1).

The method described here enables the quantitative determination of respirable suspended particles (RSP) in ETS based on the tracer solanesol. Solanesol remains in a constant ratio to RSP contributed by tobacco smoke over various ventilation conditions and sampling durations (see Ref. 3.2). Both ultraviolet particulate matter (UVPM) and fluorescent particulate matter (FPM) also fulfill this requirement (see Ref. 3.3). However, airborne solanesol is unique in that it is found only in tobacco smoke and only in the particulate phase of ETS. Solanesol's high molecular weight and low volatility also make it very unlikely that any compound will be lost from the membrane filter during sample collection. ETS RSP consists of approximately 3% solanesol by weight (see Ref. 3.4, 3.5, 3.6): appropriate for measurement at realistic smoking rates. Of all ETS particulate phase markers (UVPM, FPM, and solanesol), solanesol is considered the best marker for particulate phase determination and thus the best method for assessing the contribution of ETS particulate matter (ETS-PM) to RSP (see Refs. 3.7, 3.8, 3.9, 3.10, 3.11, 3.12, 3.13, 3.14, 3.15).

The importance in quantifying the contribution of ETS to RSP through tobacco-specific markers lies in the fact that while RSP is a necessary indicator of overall air quality, it is not specific to tobacco smoke. The Occupational Safety and Health Administration (OSHA) previously set a permissible exposure level (PEL) of respirable dust in the workplace at 5000 $\mu g/m^3$. However, since RSP can emanate from various sources (see Ref. 3.16) and has been demonstrated to be an unsuitable tracer of ETS (see Refs. 3.4, 3.17, 3.18, 3.19), a marker that is more selective must be used to determine the contribution of tobacco smoke to RSP. UVPM and FPM, while more selective than RSP and useful in indoor air quality studies, tend to overestimate the contribution of tobacco smoke to RSP due to potential interference from non-tobacco combustion sources. Solanesol, then, is the better indicator of tobacco smoke contribution to RSP. The following method apportions RSP into ETS and non-ETS components by determining the weight ratio of solanesol to total RSP (see Refs. 3.4, 3.6, 3.10, 3.11, 3.14, 3.15, 3.20, 3.21).

Many plants of the *Solanaceae* family, which includes the genus *Nicotiana*, of which the tobacco plant is a member, contain solanesol; particularly those that contain trace amounts of nicotine. These include the tomato, eggplant, potato, and pepper. The potential interference due to these sources is negligible, cooking being the only likely potential source of interference. An interference of this type would bias results high, overestimating the contribution of ETS to RSP.

2. DEFINITIONS

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

2.2. *Respirable Suspended Particles (RSP)*
The particles which, when captured by a size-selective sampling device, conform to a collection efficiency curve with a median cut point at an aerodynamic diameter of 4.0 μm.

2.3. *Environmental Tobacco Smoke Particulate Matter (ETS-PM)*
The particulate phase of ETS.

2.4. *Solanesol Particulate Matter (Sol-PM)*
An expression for the contribution of ETS-PM to RSP based on solanesol determination.

3. REFERENCES

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4. PRINCIPLE

A known volume of air is drawn through an inertial impactor or cyclone separating at 4.0 µm, separating RSP from total suspended particulate matter, followed by a filter cassette containing a polytetrafluoroethylene (PTFE) membrane filter to collect RSP and solanesol. Solanesol is desorbed into methanol, and an aliquot is injected into a high performance liquid chromatography (HPLC) system equipped with UV detection (205 nm absorbance).

5. APPARATUS

5.1. Polytetrafluoroethylene (PTFE) membrane filter with 1.0 µm pore size and 37 mm diameter, bonded to a high-density polyethylene support net, filter backing, for improved durability and handling ease.

5.2. Filter cassette, made of black, opaque, conductive polypropylene in a three-piece configuration.

5.3. Bubble flowmeter or mass flowmeter, for calibration of the sampling pump.

5.4. Personal or Constant-flow air sampling pump calibrated for a flow rate dependent upon the separating characteristics of the impactor or cyclone used.

5.5. Inertial impactor or cyclone with nominal cut point of 4.0 µm at the specified flow rate, for separating RSP from total suspended particulate matter.

5.6. HPLC system consisting of HPLC pump, UV detector with deuterium source lamp, autosampler, column oven (optional), and data acquisition and peak integration system. Recommended HPLC column: 250 mm x 3.0 mm id, Delta bond C18 (ODS) column (pore size 300 Å and particle size 5 µm) from Keystone Scientific, Bellefonte, PA, USA.

5.7. Guard cartridge column, compatible with packing material and dimensions of the HPLC column (5.6.) used in front of the analytical column, for protection and extension of the life of the column.

5.8. Sampling containers consisting of 4 ml low-actinic borosilicate glass vials with screw caps and PTFE-lined septum closures (*e.g.*, autosampler vials).

5.9. Dispensing pipets, 3.00 ml.

5.10. Filter forceps, for handling the filters.

5.11. Wrist-type action shaking device, for solvent extraction.

5.12. The necessary general laboratory equipment (volumetric flasks, pipets, syringes, etc.), for the preparation of samples, standards and reagents.

6. REAGENTS

6.1. Acetonitrile (HPLC grade), for the mobile phase of the HPLC system

6.2. Methanol (HPLC grade), for desorbing solanesol from PTFE filter, preparing standard solutions, and for the HPLC mobile phase.

6.3. Solanesol (minimum purity 90+%), for preparation of standard solutions.

6.4. Helium (minimum purity 99.995% grade), for purging the HPLC system (optional).

7. STANDARDS

7.1. Solanesol standard solutions: (1) *Primary* (*300 μg/ml*) – weigh 30 mg solanesol into a 100 ml volumetric flask and dilute to volume with methanol. (2) *Secondary* (*15 μg/ml*) – pipet 5.0 ml of the primary solution into a 100 ml volumetric flask and dilute to volume with methanol. (3) *Tertiary* (*6 μg/ml*)– pipet 2.00 ml of the primary solution into a 100 ml volumetric flask, dilute to volume with methanol, and shake to mix.

7.2. Prepare five working standard solutions *that cover the concentration range of interest* (*e.g.*, *0.6, 0.150, 0.450, 1.05, and 3.00 μg/ml*) by adding 1.0 ml tertiary, 1.0, 3.0, and 7.0 ml secondary, and 1.0 ml primary standards to 100 ml volumetric flasks. Dilute to volume with methanol and shake to mix.

7.3. All standards should be stored in low-actinic borosilicate screw-cap jars in a freezer (0 °C or lower). New standards should be prepared at least every 12 months.

8. PROCEDURES

8.1. *Air Pumping System Calibration*

Obtain the specified flow rate for the particular inertial impactor or cyclone used by adjusting the potentiometer on the air sampling pump. Calibrate the system by connecting the flowmeter to the inlet of the inertial impactor (or cyclone) and measuring the flow with the filter cassette, placed between the pump and the impactor (or cyclone). The air sampling pump should be calibrated before and after each sampling.

If a mass flowmeter is used, record the volumetric flow rate of the sampling pump.

If a bubble flowmeter is used, measure the time for a soap-film bubble to traverse a known volume with a stopwatch. Calculate mean time from at least five replicate measurements. (Be sure to allow several soap bubbles to form in the flowmeter and thoroughly wet the surface before recording any measurements). The volumetric flow rate, q_V, expressed in liters per minute (l/min), is calculated as follows:

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

where

V_s is the volume measured by the flowmeter, expressed in liters (l);

t_s is the mean time for a soap bubble to travel the known volume in the flowmeter, expressed in minutes (min).

8.2. *Sample Collection*

Upon proper insertion and positioning of the prepared filter cassettes between the impactor (or cyclone) and the sampling pump, switch the pump power on to begin sampling and document the starting time. Collect the samples at the flow rate required by the impactor (or cyclone) for at least one hour. Switch the power pump off to terminate the sampling period. Document the elapsed time for sample collection (*t*).

After sample collection, check the flow rate of the pump. Calculate the average flow rate, ($\bar{q}v$), mean of before and after sample collection. Use this average flow rate for future calculations.

Remove the filter cassette with the sample collected on the membrane filter from the sampling system. Replace the plastic plugs on the inlet and outlet ports of the cassette.

Treat at least six prepared filter cassettes with filters in the same manner as the samples to serve as field blanks.

Store all filter cassette samples in a freezer (< 0 °C) if not analyzed immediately.

Note: Analyze all the filters within six weeks after sample collection. It has been established that samples are stable for at least six weeks at –10 °C storage conditions (see Ref. 3.22).

8.3. *Sample Preparation*

Place each filter into a clean sample vial and add 3.00 ml of methanol (V_m). Prepare the field blank samples, as well as two unweighed filters (laboratory blanks) in the same manner. Tightly seal the vials with septum/cap and place in a holding tray for agitation. Shake all vials in a wrist-type action shaking device and allow to extract for 60 minutes.

8.4. *HPLC Apparatus Setup*

Prepare the apparatus and operate the high performance liquid chromatography system according to the manufacturer's instructions. The conditions for UV detection are as follows:

Mobile phase purge gas: helium (optional)
Mobile phase: 95:5 (v/v) acetonitrile:methanol
HPLC pump flow: 0.5 ml/min
Injection volume: 100 µl
Run time: 15 min
Detector wavelength setting: 205 nm
The above conditions for the analytical and guard columns should provide a retention time of approximately 9 minutes for solanesol.

8.5. *Determination*

Before analysis, allow previously frozen samples to attain room temperature equilibration for at least 60 minutes and shake vigorously to mix. Load one set of working standards into the autosampler, followed by the experimental samples, field blanks, and laboratory blanks. Follow the laboratory blanks with a second set of working standards. Acquire the integrated peak area counts for the standards, samples, and blanks through data acquisition and peak integration system. Construct a calibration curve by plotting the mean peak area of solanesol (ordinate) versus the concentration of solanesol in µg/ml (abscissa) using the working standards. Obtain the slope and y-intercept using linear regression.

8.6. *Calculations*

Using the calibration curve convert the area counts from the sample and blank injections into solanesol content (µg/ml).

Calculate the solanesol content, ρ_S, in micrograms per milliliter (µg/ml) of solution from the following equation:

$$\rho_S = \rho_{SS} - \rho_{SB} \quad (2)$$

where

ρ_{SS} is the concentration of solanesol determined by the calibration curve, expressed in micrograms per milliliter (μg/ml);

ρ_{SB} is the average concentration of solanesol of all blanks, also determined by the calibration curve, expressed in micrograms per milliliter (μg/ml).

Calculate the mass of solanesol, m_S, expressed in micrograms (μg) from the following equation:

$$m_S = \rho_S \times V_M \tag{3}$$

where

ρ_S is the concentration of solanesol determined by the previous equation, expressed in micrograms per milliliter (μg/ml);

V_M is the volume of methanol used in the extraction from the filter, expressed in milliliter (ml).

Calculate the solanesol content in sampled air, ρ_{SA}, expressed in micrograms per cubic meter (μg/m^3), from the following equation:

$$\rho_{SA} = \frac{m_S \times 1000}{t \times \bar{q}_v} \tag{4}$$

where

m_S is the mass of the solanesol, expressed in micrograms (μg), from Equation (3);

t is the time elapsed for sample collection, expressed in minutes (min);

$\bar{q}_v$ is the average flow rate, expressed in liters per minute (l/min), determined in Step 8.2;

1000 is the factor converting liters to cubic meters, expressed in liters per cubic meter (l/m^3).

Calculate the content due to ETS-PM content in the air sample, ρ_{SP}, (μg/m^3) from the following equation:

$$\rho_{SP} = \frac{\rho_{SA}}{0.0303} \tag{5}$$

where

ρ_{SA} is the solanesol content, expressed in micrograms per cubic meter (μg/m^3), determined in the air sample calculated in Equation (4);

0.0303 is the empirically determined weight ratio of solanesol, based on the fact that solanesol makes up 3.03% by weight of RSP to ETS.

Note: This conversion factor is an aggregate of factors determined empirically in an environmental test chamber where the only RSP present was that generated from the normal smoking of selected cigarettes. Individual factors include: 0.0303 (± 0.00076) determined for the leading 50 cigarette brand styles in the United States (see Ref. 3.6), 0.0230 for the leading six cigarette brand styles in each of 10 non-U.S. countries (see Ref. 3.23), and 0.0258 for the leading six cigarette brand styles in each of 8 non-U.S. countries (see Ref. 3.24). It should also be noted that, if the ETS-PM being measured is from a specific tobacco product with a known conversion factor, then this factor should be

substituted. The applicability of this ratio has not been determined for tobacco smoke not meeting the definition of ETS given in 3.1 (for example, machine-generated sidestream smoke).

Calculate the total RSP content in the air sample, ω_{ES}, the mass fraction percent, (%) attributed to the ETS particulate phase by the equation:

$$\omega_{ES} = \frac{\rho_{SP}}{\rho_{RA}} \times 100 \quad (6)$$

where

ρ_{SP} is the RSP content, expressed in micrograms per cubic meter ($\mu g/m^3$), attributed to ETS-PM from Equation (5);

ρ_{RA} is the total RSP content in the sampled air, expressed in micrograms per cubic meter ($\mu g/m^3$), (see Ref. 3.3).

9. POTENTIAL INTERFERENCE

Potential interference from the cooking of plant material from the *Solanaceae* family that may contain solanesol, including tomato, eggplant, potato, and pepper, could conceptually overestimate the contribution of ETS to RSP. However, the potential interference due to these sources is almost certainly negligible. The only airborne solanesol measurable in an indoor environment likely comes from tobacco combustion.

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 solanesol and six levels. The data from the laboratories contained two 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 2.2 µg to 7.4 µg/sample for solanesol. The 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 of a and A are listed in the following table (see Ref. 3.25).

Table 1 – Values a and A

Analyte	a	A
Solanesol	0.032	0.168

11. LIMITS AND DETECTION

The method specified allows the estimation within the following limits of solanesol content. At a sampling rate of 2 l/min, limits of detection (LOD) and quantification (LOQ) for solanesol in air are, respectively, 0.042 $\mu g/m^3$ and 0.140 $\mu g/m^3$ for a 1 h sampling period and 0.005 $\mu g/m^3$ and 0.017 $\mu g/m^3$ for an 8 h sampling period (see Ref. 3.12).