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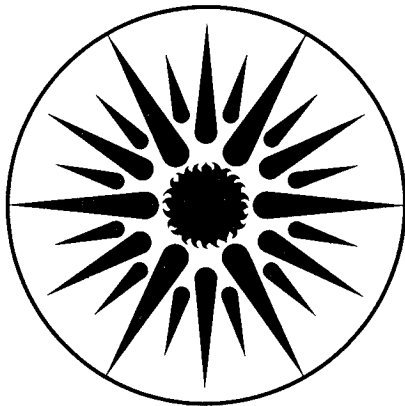
## APPLIED SCIENCE DIVISION

### **A Semi-Micro Soxhlet Extraction Method for the Determination of Extractable Particulate Organic Matter and Selected Polycyclic Aromatic Hydrocarbons**

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February 1989

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**A SEMI-MICRO SOXHLET EXTRACTION METHOD FOR THE DETERMINATION OF  
EXTRACTABLE PARTICULATE ORGANIC MATTER AND SELECTED POLYCYCLIC  
AROMATIC HYDROCARBONS**

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**February, 1989**

## ABSTRACT

A semi-micro-Soxhlet extraction method has been developed for the determination of extractable particulate organic matter and selected polycyclic aromatic hydrocarbons in samples of particulate matter collected in indoor air. This method can be applied to 25 m<sup>3</sup> samples of suspended particulate matter collected in homes in which concentrations are about 10  $\mu\text{g}\cdot\text{m}^{-3}$  or more. The method involves a sequential extraction in a micro-Soxhlet apparatus with dichloromethane (DCM) followed by an optional extraction with acetone (ACE). The extracts are then filtered in a specially developed semi-micro filtration/evaporation apparatus and brought to a final volume of 1.0 ml for extract weight determination. An aliquot of the DCM fraction is then analyzed for selected polycyclic aromatic hydrocarbons (PAH) by high pressure liquid chromatography (HPLC) using fluorescence detection for high sensitivity.

## INTRODUCTION

Indoor air sampling presents certain constraints on sampling rates which are not encountered in outdoor air sampling. The upper limit for the air sampling flow rate is constrained by consideration of the impact of the sampling on the indoor concentration of the pollutant of interest. That is, the sampling rate must be sufficiently low so that the concentration of the pollutant of interest is not lowered by the act of sampling. To minimize the impact of the sampling, the sampling flow rate should ideally be no more than about 5 % of the air exchange rate for the enclosed space of interest. The lower limit for the air sampling is constrained by the air volume required for the analytical method, i. e., sufficient material must be collected for the analysis.

Most indoor air sampling for extractable particulate organic matter (EPOM) and for polycyclic aromatic hydrocarbons (PAH) to date has involved the use of relatively high air flow rates. These have generally ranged from about  $0.57 \text{ m}^3\text{-min}^{-1}$  (Butler and Crossley, 1981) to  $0.208 \text{ m}^3\text{-min}^{-1}$  (Chuang et al., 1985). For a typical  $41 \text{ m}^3$  ( $12' \times 15' \times 8'$ ) room in a house, sampling at a flow rate of  $0.208 \text{ m}^3\text{-min}^{-1}$  is equivalent to about 30 % of an air change per hour for that room.

For the determination of EPOM and PAH, 600 to 2000  $\text{m}^3$  samples have typically been collected on 8" x 10" high-volume filters and extracted in a conventional Soxhlet apparatus with 150 to 200 ml of organic solvent. The extract volume is then reduced under vacuum using a rotary evaporator (Daisey et al., 1981; 1984; 1986). In principle, determination of EPOM requires masses of the order of micrograms since this is the minimum mass that can be weighed. Similarly, only picogram quantities of PAH are required for analysis by high-pressure liquid chromatography with fluorescence detection (Gundel et al., 1981). These quantities, however, must be present in a volume of solution which can be measured and

handled with both precision and accuracy. Furthermore, the semi-micro techniques needed for the extraction, concentration and volume measurements must be worked out in detail. In general, there has been very little work to develop the semi-micro methods which would make it feasible to sample and analyze small quantities of EPOM and PAH in indoor air. This report presents a semi-micro Soxhlet method for the determination of extractable particulate organic matter and PAH which requires a sample of particulate matter from only 25 m<sup>3</sup> of indoor air. The method is an adaptation of methods developed previously for outdoor air (Daisey et al., 1981, 1984; Gundel et al., 1981).

## OVERVIEW OF THE METHOD

The method described here is suitable for the determination of two fractions of non-volatile EPOM and selected PAH in samples of airborne particulate matter (PM) collected in homes and white-collar workplaces. The method involves a sequential extraction in a micro-Soxhlet apparatus with dichloromethane (DCM) followed by an optional extraction with acetone (ACE). An aliquot of the DCM fraction is then analyzed for selected PAH by high pressure liquid chromatography (HPLC) using fluorescence detection for high sensitivity. This method can be applied to 25-m<sup>3</sup> samples of particulate matter (PM) collected in homes in which concentrations of respirable particles are greater than about 10 µg-m<sup>-3</sup>.

The lower limits of detection (LLDs) for the two EPOM fractions depend upon the type and size of filter used since the filter makes the largest contribution to the blank correction for the extractable mass. For A 9 cm<sup>2</sup> Teflon-coated glass fiber filter, heated for 24 hours at 170°C and a 25-m<sup>3</sup> air sample, the LLDs for the DCM and ACE-soluble fractions, respectively, are 0.6 and 0.8 µg-m<sup>-3</sup>. Lower LLDs can be achieved by solvent extraction of the filters prior to sampling. The precision of the method, determined by triplicate extractions of 7- to 14-mg samples of urban particulate matter, was 7.5 % (average relative standard deviation) for the DCM fraction and 11.5 % for the ACE fraction.

Eight PAH can be determined at the fluorescence wavelengths and HPLC conditions given: fluoranthene, pyrene, benzo(e)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene). Fluoranthene and pyrene are also present in the gas phase which is not sampled here. The LLDs for PAH compounds range from 0.02 to 0.12 ng-m<sup>-3</sup> for a 25-m<sup>3</sup> sample. Precision and accuracy are 10 to 20 %.

## PRINCIPLE

Airborne particulate matter from 25 m<sup>3</sup> of air is collected on a filter. The loaded filter is extracted in a micro-Soxhlet apparatus with DCM, then (optionally) with acetone. The DCM removes the non-polar and moderately polar compounds while the ACE removes the polar compounds. Some inorganic matter may be extracted with the ACE, e.g., NH<sub>4</sub>NO<sub>3</sub>. For outdoor samples collected in summer, NH<sub>4</sub>NO<sub>3</sub> is typically 10 to 20 % of the total ACE fraction. Lower percentages would be expected for most indoor samples. The extracts are filtered and concentrated to a 1.0-ml volume. Duplicate 100 ml aliquots are taken to dryness and weighed to determine the extractable masses. Another aliquot is used for analysis of PAH using HPLC with fluorescence detection. The remaining extract may be used for other chemical analyses or for a micro-bioassay.

## HAZARDS

The mixed standard used for calibrating the HPLC, the DCM extracts of the samples and the positive controls contain carcinogens and should be handled to avoid skin contact and aerosol generation. Organic solvents should be used in a hood to prevent inhalation exposures.



## REAGENTS

Acetone	Distilled-in-glass grade.
Dichloromethane (DCM)	Distilled-in-glass grade. Check each lot of extraction solvents by evaporating 30 ml of solvent to 1.0 ml and determining residue as on p. 13
Acetonitrile	HPLC grade, peroxide-free (bottle should be freshly opened and checked for the presence of peroxides). Opened bottle should be flushed with high-purity nitrogen before re-capping.
Calibration Standards	Standard reference materials (SRM) No. 1649 (urban particulate matter) and No. 1647 (priority pollutant polynuclear aromatic hydrocarbons in acetonitrile), from the U.S. National Bureau of Standards; individual PAH (for confirmation of compound identification by co-injection).
Calibration Solutions	Prepare five or six different dilutions of SRM No. 1647 in acetonitrile, with benzo(a)pyrene concentrations ranging from 2 to 50 ng/ml. Prepare standard solutions of the individual PAH in acetonitrile at concentrations about ten-fold higher than those in SRM No. 1647. For the single set of excitation and emission wavelengths used in the method, sensitivities for benzo(ghi)perylene and indeno(1,2,3-cd)pyrene are lower than those for other PAH.
HPLC Solvents	Prepare, filter and deaerate the following: Solvent A: tetrahydrofuran: acetonitrile: water (2.5:47.5:50, v/v); Solvent B: tetrahydrofuran: acetonitrile (5:95, v/v).

## APPARATUS AND SUPPLIES

Micro-Soxhlet extractors	Overall height, ~36 cm, with 12/18 and 24/25 ground glass joints
Flasks	25-mL, round-bottomed, single-necked (for Soxhlet extractors) with 12/18 glass caps (female)
Soxhlet heating units	
Boiling beads	Glass
Teflon filters	Unlaminated, 47 mm diam., 0.45 $\mu\text{m}$ pore size
Micro-vials	2-mL, with conical inner bottoms and Teflon-lined caps (flat-bottomed, amber vials may also be used)
Syringes	1.00-mL, gas-tight, glass or Teflon, with blunt tips; 25- $\mu\text{L}$ , for HPLC injections
Micro-pipettes	100- $\mu\text{L}$ Wiretrol (Fisher Scientific, Inc., Pittsburg, PA) or 100- $\mu\text{L}$ syringe
Balance	Capable of weighing to within 0.1 $\mu\text{g}$ (e.g., Cahn Electrobalance), with disposable aluminum weighing pans
HPLC	With 25 cm $\times$ 4.6 cm C-18 5- $\mu\text{m}$ Vydac TP 201 (or equivalent) reversed-phase column and fluorescence detector (the column must separate benzo(a)pyrene from benzo(k)fluoranthene and benzo(ghi)perylene from indeno(1,2,3-cd)pyrene). Use Waters Associates Inc., Curve No. 9 for baseline check.
Freezer	Explosion-proof, for sample storage at -30 $^{\circ}\text{C}$ .
Aluminum foil	
Sonicator bath	
Filtration/evaporation apparatus	Consists of a 10-mm diam., medium porosity, fritted glass filter stick (for gas dispersion) with a flat frit and a 10-cm $\times$ 7-mm

diam. stem (Reliance Glass, Bensenville, IL), plus a 25-mL, side-arm test tube or a 125-mL side-arm vacuum flask. Insert stem of fritted-glass filter through a foil-lined stopper and place stopper into neck of test tube (or flask) so that stem of filter may be inserted into top part of sample collection vial (see 9.5.2)

#### Air sampler

The EPA modified high-volume sampler developed by Lewis and Jackson (1982) can be used, or samples may be collected at 20 L/min on 47-mm diam., Pallflex, Teflon-coated, glass-fibre filters.

### SPECIAL PRECAUTIONS

All loaded filters and extracts should be protected from light at all times or handled in a laboratory equipped with yellow lights.

The THF used for HPLC analyses should be freshly opened and/or checked for the presence of peroxides. After opening, the bottle should be flushed with high purity nitrogen gas and tightly capped.

### SAMPLING

This semi-micro method was developed using 25-m<sup>3</sup> aliquots of indoor samples of respirable suspended particulate matter (RSP) collected with a modified high-volume sampler at a flow rate of 425 l-min<sup>-1</sup> (Daisey et al., 1987). For indoor air sampling, a lower flow rate is recommended in order to minimize the impact of the sampling on the indoor concentrations being measured. Samples of particulate matter, collected at a flow rate of 20 l-min<sup>-1</sup> for 24 hours (about 25 m<sup>3</sup>) on 47-mm diameter Pallflex Teflon-coated glass fiber filters, also provide sufficient particulate material for this method. The filters are cleaned prior to use by Soxhlet extraction with DCM. After weighing, wrap each filter in solvent-rinsed or fired (heated in a

muffle oven at 400 °C) aluminum foil and label the foil packet. If part of the sample is to be analyzed for organic and elemental carbon, a prefired (750 °C) quartz filter should be used. After sample collection, fold the loaded filter in half, with the particulate-loaded side facing in, wrap again in the aluminum foil and store in a freezer at -30 °C until extraction. Storage time should be two weeks or less.

## **PROCEDURES FOR THE DETERMINATION OF EPOM**

### **BLANKS AND CONTROLS**

All glassware must be cleaned to reduce organic contamination. Non-volumetric glassware may be heated in a muffle oven at 400 °C for 24 hours. Other glassware should be cleaned with detergent followed by a potassium hydroxide/ethanol wash. Rinse copiously with tap water and then rinse five times with deionized water. Immediately prior to use, all glassware should be rinsed twice with distilled-in-glass solvent. The residue for each lot of extraction solvent should be checked by reducing 30 ml of solvent to 1.0 ml and determining the residue (See extract weight determination).

Extracts should be stored in 2.0-ml micro vials with conical inner bottoms and Teflon-lined caps. Solvent-rinsed aluminum foil should be used as a liner between the glass and Teflon-lined cap to minimize contamination from the materials under the Teflon lining.

Field blank filters (ten percent of sample number) should be extracted and analyzed at the same time and in the same manner as the samples to determine the filter-solvent blank correction. Ten-milligram samples of U.S. National Bureau of Standards Standard Reference Material No. 1649 (urban particulate matter) should also be wrapped in filters, extracted and analyzed as positive controls.

## EXTRACTION

1. Add 15 ml of DCM and a clean glass boiling bead to each flask. Place a label with the sample identification on the neck of each flask.
2. Fold each sample, blank and control filter (or portion of one), loaded side in, and insert in the Soxhlet tubes using cleaned tweezers. An internal standard may first be spiked onto the filter. The filter must be completely below the siphon. Label the each Soxhlet tube with the sample identification number.
3. Assemble the flask, micro-Soxhlet tube, and condenser and place in a heating mantle or on a Soxhlet heating unit. **DO NOT GREASE THE GROUND JOINTS.** Make sure that the water is flowing through the condenser and that the condenser remains cold throughout the extraction.
4. Turn on the heaters and adjust to cycle once every 10 minutes. Cover the extractors and flasks with aluminum foil to protect from light. Continue the extraction for 7 hours.
5. At the end of the extraction period, at a point at which the Soxhlet chamber is almost filled with solvent, turn off the heaters and insert an insulator between the flask and the heater or raise the flask from the heating mantle to cool. Separate the flask and Soxhlet tube from the condenser and then from each other and carefully decant any solvent remaining in the Soxhlet tube into a waste disposal container. About 5 to 10 ml of extract should remain in each of the flasks. Cap the flasks with a ground glass standard taper cap. If the samples are to be extracted with acetone, wrap the Soxhlet tubes in aluminum foil and store in a freezer overnight.
6. A second extraction with ACE is done in the same manner. Once the second extraction is

begun, the DCM extracts from the previous day are thawed and filtered.

## FILTRATION AND EVAPORATION

1. Bring the extracts to room temperature.
2. Assemble the filtration/evaporation system. Insert the 2-ml micro-vial into the bottom of the side-arm test tube with metal tweezers (cleaned with an acetone rinse). Insert the stem of the filter stick about 1 cm into the opening of the vial so that the filtered extract drops into the vial. The end of the stem must be above the solution when the filtration is completed.
3. Set up the filtration/evaporation system with a glass trap between the filtration tube and the aspirator or vacuum line using glass and/or Teflon tubing for connections. Place the trap in an ice bath. Place the side-arm test tube in a warm-water bath to minimize condensation of atmospheric water in the test tube and clamp in place. (A valve may be placed between the filtration apparatus and the trap for better control of the vacuum.)
4. Wrap a 47-mm diameter (0.45- $\mu$ m pore) Teflon filter over the glass frit of the filtration/evaporation apparatus and press the overlapping portion against the stem with a slight twisting motion. Avoid touching the center of the Teflon filter with fingers. With the vacuum on, rinse the filter and vial twice with distilled-in-glass grade DCM and discard rinsings.
5. Fill the 1.0-ml syringe to about one-third with the extract in the 25-ml flask. With the vacuum on, carefully transfer the extract to the filtration system and transfer the extract to the center of the filter with a slow steady motion so that there is no extract spillover, i.e., the extract is immediately drawn through the filter. Repeat this transfer step until all

of the extract has been filtered.

6. When all of the extract has been filtered, rinse the 25-ml flask with about 0.2 to 0.3 ml of the appropriate solvent (DCM or ACE), draw the rinsings into the syringe, rinse the syringe and filter the rinsings into the 2-ml vial with the extract. Repeat this twice more.
7. Keep the filtrate under vacuum until the total volume is reduced to about 0.5 ml. Much of the solvent will evaporate during the filtration step.
8. Break the vacuum at the side-arm of the test tube. With the tweezers, carefully remove the vial from the bottom of the side-arm test tube and place in a small beaker or holder for stability. Loosely cover the top of the vial with cleaned aluminum foil and allow the vial and extract to come to room temperature.
9. Draw the extract into a cleaned 1.0 ml syringe. To remove any air bubbles, draw more air into the syringe to create an air bubble with a diameter larger than that of the needle. Holding the syringe vertically, slowly and carefully expel the air bubble through the needle until the extract just reaches the end of the needle. Add a small amount of solvent to the vial to rinse it, then draw this into the same syringe. Repeat if necessary. Bring the total volume in the syringe to 1.00 ml, then transfer the extract back into the same 2.0 ml micro-vial. The micro-vial should be dry. If necessary, dry with a stream of high purity nitrogen before returning the extract.
10. Cap the vial with a solvent-rinsed piece of aluminum foil, then with the Teflon-lined cap. Label the vial and make a mark on the side (using a diamond glass marker or a pen mark on an adhesive label) to indicate the meniscus of the extract. Store in a freezer.

## DETERMINATION OF EXTRACT WEIGHTS

1. Remove the extract samples from the freezer, bring to room temperature and sonicate for 20 to 30 seconds in a sonicator bath. Check the solution volume against the mark on the vial. If there has been any solvent loss, bring the volume up to the mark and sonicate again.
2. Adjust a slide warmer or hot plate to 35-40 °C and place in a hood next to the balance.
3. Tare a disposable aluminum weighing pan on the Cahn Electro-balance and then transfer the pan to the slide warmer.
4. Rinse a clean 100  $\mu$ l Wiretrol (or syringe) first with solvent and then with the extract. Fill the Wiretrol to the mark, wipe the end of the Wiretrol carefully, then transfer the 100  $\mu$ l of extract to the weighing pan.
5. Evaporate to dryness on the slide warmer. This usually takes about 5 minutes.
6. Weigh the residue. If all of the solvent has evaporated, a constant weight (about  $\pm 0.2 \mu$ g) will be obtained. Highly concentrated and sticky extracts must sometimes be diluted to obtain a constant weight.
7. Repeat the weight determination with a second 100- $\mu$ l aliquot. The weights should differ by no more than about  $\pm 10\%$ . If necessary, dry and weigh a third aliquot. Blanks sometimes do not meet this criterion because of the low weights.



## METHOD OF CALCULATION FOR EXTRACTABLE PARTICULATE ORGANIC MATTER

1. Calculate the net extract weights for the two aliquots of extract and determine the average weight.
2. Subtract the average blank.
3. 
$$\mu\text{g EPOM}/\text{m}^3 = \frac{(\text{mg in } 100 \mu\text{l}) \times 10^3 \mu\text{g}/\text{mg} \times 1.0/0.1}{\text{Total m}^3 \text{ of air sampled}}$$

## METHOD FOR THE DETERMINATION OF SELECTED POLYCYCLIC AROMATIC HYDROCARBONS

1. Connect a 25-cm  $\times$  4.6-cm C-18 Vydac 201 TP 5- $\mu\text{m}$  HPLC column and operate the HPLC according to the manufacturer's instructions.
2. Set the HPLC fluorometer detector at 280 nm and  $>389$  nm for the excitation and emission wavelengths, respectively. To help confirm compound identities, selected samples should also be analyzed at a different set of excitation and emission wavelengths and the peak height ratios for the two sets of fluorescence conditions should be compared to those of standards.
3. Check the baseline for the column using the following program: 2.0 ml/min; 25% solvent B to 95% solvent B over 35 minutes using Waters Associates, Inc., Curve #9 (non-linear), hold for 10 minutes or more, as required to return to near baseline fluorescence. Reverse the gradient to initial conditions (10 minutes); hold at initial conditions for 10 minutes. These same times should be used for each analysis to assure reproducible retention times.

4. Prepare calibration curves for the PAH by injecting and analyzing 25- $\mu$ l aliquots of the diluted PAH calibration solutions. Use co-injections of a solution of a single PAH with the mixed PAH standard to help confirm compound identification.
5. For some instruments, the lamp output will drift throughout the day. Column conditions will also change. Overall drift should be determined by repeated injections of the same standard over an 8-hour period. If peak heights for any PAH change by  $\pm 15\%$  or more over the 8 hours, correct for changes in the lamp output and column condition during the day of analysis as follows.

Note the clock time of each HPLC injection and record. The first (at  $t_0$ ) and last (at  $t_f$ ) injections of the day should be a mixture of the standards (same concentration). Then the peak height measured at time =  $t$ ,  $P(t)$ , is corrected back to  $t_0$  according to:

$$P(t_0) = P(t) \times C(t),$$

where  $P(t_0)$  and  $P(t)$  are the peak heights of the compound in the sample at times  $t_0$  and  $t$ , respectively, and  $C(t)$  is the correction factor for time  $t$ .

$$C(t) = S(t_0)/S(t).$$

$S(t)$  is calculated according to:

$$S(t) = S(t_0) - \{[S(t_0) - S(t_f)] [(t-t_0)/(t_f-t_0)]\}.$$

where  $S(t_0)$ ,  $S(t)$  and  $S(t_f)$  are the peak heights of the standards at times  $t_0$ ,  $t$  and  $t_f$ , respectively. The expression in brackets is the amount that the standard peak height would have changed during the time interval  $t-t_0$ , based on a linear rate of change during

the time interval  $t_f - t_0$ .

6. Plot corrected peak heights versus pg injected and determine the slope and intercept of each calibration curve using least squares analysis. Note any non-linearity at high concentrations. If necessary, fit the calibration curve to a polynomial function,  $y = a + bx + cx^2$ , where  $y = \text{pg injected}$  and  $x = \text{peak height}$ .
7. Once the calibration curves are prepared, two injections of the mixed PAH standard should be analyzed daily with the samples.
8. Inject 25- $\mu\text{l}$  aliquots of each sample for HPLC analysis. If the fluorescence is too high, a smaller aliquot (10  $\mu\text{l}$ ) of undiluted sample extract may be injected or the sample can be diluted.
9. Determine the peak heights for each compound (identified by retention time) and use the calibration curves to calculate the pg injected in the 25- $\mu\text{l}$  aliquot.
10. Analyze the filter-solvent blanks in the same manner as the samples. In general, the blanks show little or no fluorescence and no blank correction is required unless there has been some contamination during sample handling.
11. Calculations

$$\text{pg PAH/25 } \mu\text{l injected} = \text{ng PAH/25 ml}$$

$$\text{ng PAH/m}^3 = \frac{\text{ng PAH/25 ml} \times \text{D.F.} \times 1.0 \text{ ml}}{\text{Total m}^3 \text{ of air sampled}}$$

where D.F. = dilution factor.

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