1-NITROPYRENE IN DIESEL PARTICULATES 2560

$C_{16}H_9NO_2$ MW: 247.26   CAS: 5522-43-0   RTECS: UR2480000

METHOD: 2560, Issue 1	EVALUATION: PARTIAL	Issue 1: 15 March 2003

OSHA : no PEL
NIOSH: no REL
ACGIH: no TLV

PROPERTIES: solid; d 1.246 g/cc @ 20 °C; MP 154-157 °C; BP 231 °C

SYNONYMS: None

SAMPLING		MEASUREMENT	
SAMPLER:	FILTER (Glass Fiber, 37-mm)	**TECHNIQUE:**	GAS CHROMATOGRAPHY, NITROGEN CHEMILUMINESCENCE DETECTOR
FLOW RATE:	1.0 to 2.0 L/min	**ANALYTE:**	1-Nitropyrene
VOL-MIN: -MAX:	480 L 960 L	**DESORPTION:**	1 mL toluene; place on a rotary shaker for minimum of 12 hours.
SHIPMENT:	Ship in container at ambient temperature.	**INJECTION VOLUME:**	20 µL
SAMPLE STABILITY:	At least 28 days @ 25 °C	**TEMPERATURE -INJECTION:** -DETECTOR: -COLUMN:	250 °C Furnace: 850 °C Initial: 100 °C, ramp at 7.5 °C/min to 305 °C
BLANKS:	2 to 10 field blanks per set		
ACCURACY		**CARRIER GAS:**	Helium @ 10.0 mL/min (splitless)
RANGE STUDIED:	Generated atmospheres not studied	**COLUMN:**	Capillary, fused silica, 30m x 0.53-mm ID; 1.5 µm film 5% diphenyl-95% dimethyl polysiloxane.
BIAS:	Not determined	**CALIBRATION:**	Liquid standard solutions of 1-nitropyrene in toluene.
OVERALL PRECISION (Ŝ_rT):	Not determined	**RANGE:**	0.020 to 50 µg per sample
ACCURACY:	Not determined	**ESTIMATED LOD:**	0.010 µg per sample (see Evaluation of Method)
		PRECISION (š_r):	0.02 @ 1 to 50 µg per sample

APPLICABILITY: The working range is 0.021 to 104 µg/m³ for a 500 L sample. An appropriate range of calibration standards should be used to bracket the expected analyte concentration range. Because of sensitivity and accuracy considerations, a single calibration curve will not be appropriate for the entire working range. This method was developed to analyze 1-nitropyrene in a diesel particulate matrix (see Figure 2).

INTERFERENCES: None identified - however, compounds with a similar retention time may interfere. Positive identification may be confirmed by dual column chromatography using an appropriate alternative capillary column. Identification can also be confirmed by mass spectrometry.

OTHER METHODS: None identified.

REAGENTS:

1. Toluene, HPLC or GC grade.*
2. NIST, SRM 1650, Diesel Particulate Matter.
3. 1-Nitropyrene Standard Solution, 5000 µg/mL in toluene. (AccuStandard Inc., New Haven, CT, or equivalent).*
4. 1-Nitropyrene Standard Solution, 1000 µg/mL in toluene. (AccuStandard Inc., New Haven, CT, or equivalent).*

    * See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Glass fiber filter (Type A/E) 1-µm pore size, 37-mm diameter (Gelman Sciences, Ann Arbor, MI, or equivalent), backed by a cellulose support pad 37-mm OD, in a two part, 37-mm cassette filter holder.
2. Spacer Ring, 37 mm (SKC, Eighty Four, PA or equivalent).
   NOTE: For calibration and QC samples, filter spacer rings are used instead of a support pad.
3. Personal sampling pump capable of operating for 8 hr at 2 L/min, with flexible connecting tube.
4. Labquake Rotary Shaker or equivalent.
5. Vials, Autosampler, glass with PTFE-lined crimp top cap, 1.8 mL.
6. Micro volume inserts for autosampler vials.
7. Forceps.
8. Syringes, glass: 5-µL, 10-µL, 1-mL, other sizes as needed.
9. Syringe filter with tube tip, 4-mm, 0.45-µm, PVDF.
10. Syringe, disposable: 1-mL volume.
11. Gas Chromatograph: HP-5890 with HP-7673 Autosampler (or equivalent), Antek Model 704E Nitrogen Chemiluminescence Detector (or equivalent). Restek Rtx-5-ms®, 30 m X 0.32 mm id., 1.0-µm film thickness (or equivalent).

SPECIAL PRECAUTIONS: 1-Nitropyrene is a cancer suspect agent and mutagen. Toluene is a flammable liquid, toxic.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Sample at an accurately known flow rate of 1.0 to 2.0 L/min for a volume of 480 to 960 L.
3. After the sample is collected, place the plastic plugs in the tubing connector stubs on the sampling cassette.

SAMPLE PREPARATION:

4. Extract Filters:
   a. Remove the filter from the cassette with a pair of forceps, discard the support pad, fold the filter and transfer to a GC autosampler vial.
   b. Add 1 mL of toluene and cap the vial with a septum crimp top.
   c. Place the vial on the rotary shaker. Rotate the samples for a minimum of 12 hours.
5. Prepare the filter extract for analysis.
   a. Carefully remove the septum cap from the vial containing the extracted filter.
   b. Using a transfer pipette, remove as much liquid extract from the extraction vial as possible and transfer to a 1 mL disposable syringe with a 4-mm syringe filter attached.
   c. Filter the extract into a micro volume insert in a GC autosampler vial. Cap the vial with a septum crimp cap.

CALIBRATION AND QUALITY CONTROL:

6. Calibrate daily with at least six calibration standards to cover the concentration range of the samples. A typical gas chromatogram is shown in Figure 1.
    a. Prepare calibration standards by adding known amounts of the 1-nitropyrene standard solution or a secondary stock solution prepared from the standard solution to a GC autosampler vial containing 1 mL toluene.
    b. Analyze with samples and blanks (steps 9 & 10).
    c. Prepare a calibration graph (peak area vs. μg 1-nitropyrene).
7. Determine the recovery (R) of the analyte from the sampling media using the glass fiber filters with analyte spikes in the concentration range of the samples (see step 6). Prepare three filters at each of five levels plus three media blanks.
    a. Place a filter spacer ring in the bottom section of a two part 37 mm filter cassette. Place a 37 mm glass fiber filter on top of the spacer ring (do not use a support pad).
    b. Inoculate the filter with a known amount of calibration stock solution with a microliter syringe, place the top section of the cassette in place and seal the filter in place by squeezing the two sections together.
    c. Place the plugs in the inlet and outlet tubing stubs and set the cassettes aside for a minimum of twelve hours.
    d. Prepare the filter samples (steps 4 & 5) and analyze with the calibration standards.
    e. Prepare a graph of R vs. μg 1-nitropyrene.
8. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph and R graph are in control.

MEASUREMENT:

9. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 2560-1. Inject sample with autosampler or by manual injection.
    NOTE: If peak area is above the linear range of the calibration standards, dilute the sample with toluene, reanalyze, and apply the appropriate dilution factor in calculations.
10. Measure peak area of 1-nitropyrene peak and determine μg per sample from calibration graph.

CALCULATIONS:

11. Determine the mass, μg (corrected for R) for the sample (W), and average media blank (B).
12. Calculate concentration, C, of 1-nitropyrene in the air volume sampled, V(L):

$$ C = \frac{(W - B)}{V} , mg \, / \, m^3 $$

NOTE: mg/L = mg/m^3

EVALUATION OF METHOD

This method was not evaluated with laboratory generated air samples. All of the recovery, storage studies, and LOD/LOQ determinations have been performed with laboratory spiked filters for the development of this method.

The method LOD and LOQ as determined using spiked media samples were 3.4 ng/sample and 11.5 ng/sample respectively. However, because the detector was operating at its maximum sensitivity settings, the normal baseline noise at the lowest analyte levels often made the peak difficult to be properly integrated. Therefore, more realistic and repeatable values for the LOD and LOQ are 10 and 20 ng/sample respectively.

Average recoveries of 1-nitropyrene from spiked glass fiber filters ranged from 88.4% to 101.2% for analyte quantities of 1.0 to 50.0 µg/filter using liquid standards as the spiking solution. Recovery of 1-nitropyrene from NIST SRM-1650 Diesel Particulates weighed on glass fiber filters and carried through the extraction and sample preparation process yielded recoveries of 73% to 114% (see NOTE) for sample quantities of 1.63 mg to 26.6 mg.

A thirty day storage stability study of 1-nitropyrene spiked on glass fiber filters indicates that the recovery of the analyte at a level of 10 µg/filter is 102%. The samples were stored in a closed cardboard container at room temperature for the duration of the study.
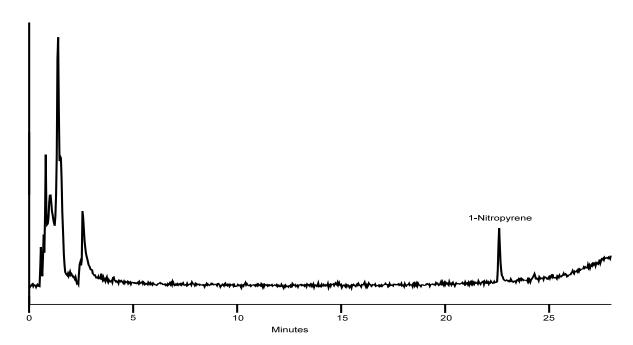
NOTE: The rated concentration of the NIST SRM-1650 for 1-Nitropyrene is 19$\pm$2 ug/g diesel particulate. The uncertainty in the amount is $\pm$10.5%. This accounts for the wide range of recoveries of the NIST standard, particularly at the low amounts used in the recovery studies which were used to mimic the real world samples.
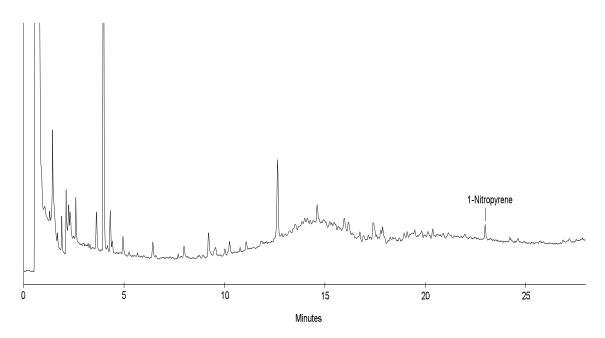
REFERENCES:

[1]   IARC [1984]. 1-Nitropyrene. IARC Monograph on Evaluation of Cancer Risk of Chemicals to Humans, *33*:209-22, Apr.
[2]   IARC [1997]. 1-Nitropyrene. IARC Monograph on Evaluation of Cancer Risk of Chemicals to Humans, *46*:321-358, Apr.
[3]   NIST [1991]. "Certificate of Analysis." Standard Reference Material 1650, Diesel Particulate Matter. Gaithersburg, MD: National Institute of Standards and Technology, December 12.
[4]   Jaycox LB [2002]. Backup Data Report, 1-Nitropyrene. Cincinnati, OH: National Institute for Occupational Safety and Health, DART. (unpublished report).

METHOD WRITTEN BY:

Larry B. Jaycox, Ph.D., NIOSH/DART

FIGURE 1: This is a chromatogram of a 1-nitropyrene 60 ng standard. The major peak at approximately 22.5 minutes is 1-nitropyrene. This chromatogram shows some hydrocarbon response and a baseline with a higher than normal noise level due to the effort to obtain as much sensitivity as possible for samples in the low nanogram region.

FIGURE 2: The chromatogram shown here is from the analysis of the NIST SRM-1650 Diesel Particulates. The diesel particulate, 2.10 mg, was weighed on a glass fiber filter and carried through the complete sample extraction and preparation procedure. The quantity of 1-nitropyrene in this sample was 39.9 µg.