Aniline C_6H_7N	MW: 93.13	CAS: 62-53-3	RTECS: BW6650000
o-Toluidine C_7H_9N	MW: 107.2	CAS: 95-53-4	RTECS: XU2975000
Nitrobenzene $C_6H_5NO_2$	MW: 123.1	CAS: 98-95-3	RTECS: DA6475000

METHOD: 2017, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 January 1998**

OSHA: Table 1
NIOSH: Table 1
ACGIH: Table 1

PROPERTIES: Table 1

SYNONYMS: Aniline: aminobenzene, benzenamine, phenylamine
o-Toluidine: *o*-aminotoluene, 2-aminotoluene, 1-methyl-2-aminobenzene, *o*-methylaniline, 2-methylaniline
Nitrobenzene: nitrobenzol, oil of mirbane

SAMPLING

SAMPLER: FILTER + SOLID SORBENT (glass fiber filter, sulfuric acid treated + silica gel sorbent tube, 520/260 mg)
Alternate samplers: gauze wipes, 4 x 4 in.; dermal badge sampler (1 g silica gel in cotton pouch)

FLOW RATE: 0.2 L/min [1]

VOL-MIN: 5 L
-MAX: 50 L

SHIPMENT: routine

SAMPLE STABILITY: aniline and *o*-toluidine 7 days @ 5 °C [2]; nitrobenzene 60 days [1]

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: Table 2

BIAS: not determined

OVERALL PRECISION ($\hat{S}_{rT}$): not determined

ACCURACY: not determined

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY, FID

ANALYTES: aniline, *o*-toluidine, nitrobenzene

DESORPTION: 2 mL ethanol

INJECTION VOLUME: 1 µL

TEMPERATURE-INJECTION: 250 °C
-DETECTOR: 300 °C
-COLUMN: 35 °C to 150 °C (8 °C/min)

CARRIER GAS: helium, 2.4 mL/min

COLUMN: capillary column, 30 m, 0.32-mm ID,1-µm film, (Rtx-5, or equivalent) [1]

CALIBRATION: solutions of analytes in ethanol

RANGE: Table 2 [1]

ESTIMATED LOD: Table 2 [1]

PRECISION ($\bar{S}_r$): Table 2 [1]

APPLICABILITY: Under the GC parameters stated in the method, aniline, *o*-toluidine, and nitrobenzene can be identified based upon retention time and quantified. Analyte recovery was optimized under conditions of moderate relative humidity (< 53%), low sampling rates (0.2 L/min), and sample volumes < 50 L [1]. Sulfuric acid-treated glass fiber filters were used to collect aniline and *o*-toluidine, while nitrobenzene was collected on the silica gel sorbent [1]. Nitrobenzene collection on silica gel tubes was significantly reduced under conditions of high relative humidity [1]. Dermal badge samplers (cotton pouch with 1 g silica gel) and gauze wipes were developed to estimate exposures via splashes, spills, and passive collection of aerosol vapors.

INTERFERENCES: No specific interferences were identified. However, any compound with a similar retention time may interfere.

OTHER METHODS: This method revises and combines methods 2002 (aniline and *o*-toluidine) [3], and 2005 (nitrobenzene) [4].

REAGENTS:

1. Aniline, reagent grade.*
2. *o*-Toluidine, reagent grade.*
3. Nitrobenzene, reagent grade.*
4. Ethanol, chromatographic grade.*
5. Sulfuric acid (H_2SO_4), reagent grade,* 0.26 *N*. Dilute 1.5 mL of 36 *N* H_2SO_4 to 200 mL with deionized water.
6. Aniline calibration stock solution, 102.2 mg/mL. Dilute 1 mL aniline to 10 mL with ethanol.
7. *o*-Toluidine calibration stock solution, 100.6 mg/mL. Dilute 1 mL *o*-toluidine to 10 mL with ethanol.
8. Nitrobenzene calibration stock solution, 120.3 mg/mL. Dilute 1 mL nitrobenzene to 10 mL with ethanol.
9. Helium, purified.
10. Hydrogen, prepurified.
11. Air, filtered.

*See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampling train:
   Filter, glass fiber, 37-mm (Gelman, or equivalent) treated with 0.26 *N* H_2SO_4 (see APPENDIX) in polystyrene cassette. Filters are commercially available (SKC, Inc. #225-9004, or equivalent).
   Sorbent tube, glass tube, 11 cm, 8-mm OD, containing two sections of silica gel (front = 520 mg/back = 260 mg) separated and retained by polyurethane foam plugs. A silylated glass wool plug precedes the front section. Tubes are commercially available (SKC, Inc. #226-15, Supelco ORBO-507).
2. Personal sampling pump, 0.2 L/min, with flexible connecting tubing.
3. Gas chromatograph, flame ionization detector, integrator, and column (page 2017-1).
4. Ultrasonic bath.
5. Vials, autosampler, with PTFE-lined caps.
6. Vials, 4-mL, with screw caps.
7. Microliter syringes, 10-µL and other sizes as needed, readable to 0.1 µL.
8. Flasks, volumetric, various sizes.
9. Pipets, various sizes.

SPECIAL PRECAUTIONS: Aniline is toxic, poisonous and a potential human carcinogen. *o*-Toluidine is toxic and a potential human carcinogen. Nitrobenzene is toxic and poisonous. Nitrobenzene has a synergistic effect on the toxicity of aniline and *o*-toluidine [5]. Ethanol is flammable and a dangerous fire risk. Wear appropriate protective clothing and work with these compounds in a well ventilated hood.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Assemble sampling train. Attach filter cassette to sorbent tube and to personal sampling pump with flexible tubing.
3. Sample at an accurately known flow rate (0.2 L/min) for a total sample size of 5 to 50 L.
   NOTE: High relative humidity reduces the sampling capacity of the silica gel sorbent.
4. Separate the cassettes and tubes, cap, and pack securely for shipment.

SAMPLE PREPARATION:

5. Place front (include glass wool plug) and back sorbent sections of the sampler tube in separate 4-mL vials. Discard foam plugs. Remove glass fiber filters from cassettes and place in 4-mL vials.
6. Add 2 mL of ethanol to each vial and cap.
7. Place vials in an ultrasonic bath for 60 min to aid desorption.
8. Transfer 1-mL aliquot to autosampler vials and attach crimp caps.

CALIBRATION AND QUALITY CONTROL:

9. Calibrate daily with at least six working standards over the range of interest.
   a. Add known amounts of calibration stock solutions to ethanol in 10-mL volumetric flasks and dilute to the mark.
   b. Analyze together with samples and blanks (steps 12 and 13).
   c. Prepare calibration graph (peak area vs. µg analyte).
10. Determine recovery (R) at least once for each lot of glass fiber filters and desorption efficiency (DE) for each lot of silica gel sorbent tubes used for sampling in the calibration range (step 9).
   a. Prepare three samplers at each of six levels plus three media blanks.
   b. Inject a known amount of calibration stock solutions directly onto the sulfuric acid-treated, 37-mm glass fiber filters and onto the front sorbent bed of each silica gel tube.
   c. Allow the filters and tubes to air equilibrate for several minutes, then cap the ends of the tubes and cassettes and allow to stand overnight.
   d. Desorb (steps 5 through 8) and analyze together with standards and blanks (steps 12 and 13).
   e. Prepare a graphs of R and DE vs. µg analyte recovered.
11. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration, R, and DE graphs are in control.

MEASUREMENT:

12. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 2017-1. Inject a 1-µL sample aliquot manually using solvent flush technique, or with an autosampler.
    NOTE: If peak area is above the linear range of the working standards, dilute with ethanol, reanalyze, and apply the appropriate dilution factor in the calculations.
13. Measure peak areas.

CALCULATIONS:

14. Determine the mass, µg (corrected for R), for each analyte found on the glass fiber filter (W_{Fil}) and glass fiber filter blank (B_{Fil}).
15. Calculate concentration, C_{Fil}, for each analyte in the air volume sampled, V (L):

$$C_{Fil} = \frac{W_{Fil} - B_{Fil}}{V}, \text{ mg/m}^3.$$

    NOTE: Use this equation to calculate wipe samples and dermal badges, if collected.
16. Determine the mass, µg (corrected for DE), for each analyte found in the sample front (W_f) and back (W_b) silica gel sections, and in the average media blank front (B_f) and back (B_b) sorbent sections.
    NOTE: If $W_b > W_f/10$, report breakthrough and possible sample loss.
17. Calculate concentration, C_{SG}, of each analyte in the air volume sampled, V (L):

$$C_{SG} = \frac{W_f + W_b - B_f - B_b}{V}, \text{ mg/m}^3.$$

    NOTE: µg/L = mg/m^3.

EVALUATION OF METHOD:

The method was developed in response to a health hazard evaluation (HHE) request at a rubber tire manufacturing plant where there was a reported high incidence of bladder cancer [5,6]. The method was evaluated for the collection of aniline, *o*-toluidine, and nitrobenzene on gauze wipes, passive dermal samplers, and 37-mm glass fiber filter/silica gel sorbent tube sampling train.

Desorption efficiency (DE) for aniline (recovered from acid-treated filters) was determined to be 1.00 at 10 × LOD [1]. Aniline storage stability at 0.12 mg was 64% after 7 days [1]; however, at 0.2 to 0.7 mg recovery was 93.8% [2]. (Recoveries for storage stability samples must be 90% to be acceptable.) Aniline recovery from gauze wipes spiked and air equilibrated for 8 hours was 83% with a precision of 0.048, while recovery from passive dermal samplers, spiked and air equilibrated for 1 hour, was 88% [1].

Desorption efficiency (DE) for *o*-toluidine (recovered from acid treated filters) was determined to be 1.00 at 10 × LOD [1]. *o*-Toluidine storage stability at 0.6 to 2.5 mg was 96.3% after 7 days [2], but at 0.1 mg was 76% [1]. *o*-Toluidine recovery from gauze wipes spiked and air equilibrated for 8 hours was 88% with a precision of 0.039, while recovery from passive dermal samplers, spiked and air equilibrated for 1 hour, was 95% [1].

Desorption efficiency (DE) for nitrobenzene (recovered from silica gel tubes) was determined to be 98% at 10 × LOD [1]. Nitrobenzene storage stability at 0.12 mg was acceptable after 60 days with a 95% recovery [1]. Nitrobenzene recovery from gauze wipes, spiked and air equilibrated for < 1 hour, was 89% with a precision of 0.092, while recovery from passive dermal samplers spiked and air equilibrated for 24 hours was > 92% [1].

REFERENCES:

[1] Pendergrass SM [1994]. An approach for estimating workplace exposure to *o*-toluidine, aniline, and nitrobenzene. Am Ind Hyg Assoc J *55*:733–737.
[2] NIOSH [1977]. Documentation of NIOSH Validation Tests, S168, S217, S310. Cincinnati, OH: National Institute for Occupational Safety and Health, DHEW (NIOSH) Publication No. 77-185.
[3] NIOSH [1994]. Aromatic Amines: Method 2002. In: Eller PM, Cassinelli ME, eds. NIOSH Manual of Analytical Methods, 4th ed. Cincinnati, OH: National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 94-113.
[4] NIOSH [1994]. Nitrobenzenes: Method 2005. In: Eller PM, Cassinelli ME, eds. NIOSH Manual of Analytical Methods, 4th ed. Cincinnati, OH: National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 94-113.
[5] Ward E, Carpenter A, Roberts D, *et al.* [1991]. Excess number of bladder cancers in workers exposed to ortho-toluidine and aniline. J Natl Cancer Inst *83*(7):501–506.
[6] NIOSH [1990]. NIOSH Alert: preventing bladder cancer from exposure to *o*-toluidine and aniline. Cincinnati, OH: National Institute for Occupational Safety Health, DHHS (NIOSH) Publication No. 90-116; NTIS No. PB-91-188-953.

METHOD WRITTEN BY:

Stephanie M. Pendergrass, NIOSH/DPSE

TABLE 1. EXPOSURE LIMITS AND PHYSICAL PROPERTIES

Analyte	OSHA PEL (ppm)	NIOSH REL (ppm)	ACGIH TLV (ppm)	Physical Properties
Aniline	5	Ca; lowest feasible	2	Liquid; d 1.022 g/mL @ 20 °C; BP 184°C; VP 0.089 kPa (0.3 mm Hg) @ 20 °C; MP −6 °C; explosive limits 1.3 to 11% in air.
o-Toluidine	Ca; 0.02	Ca; lowest feasible (skin)	Ca; 2	Liquid; d 1.006 g/mL @ 20 °C; BP 201 °C; MP −15 °C; VP 0.043 kPa (0.32 mm Hg) @ 20 °C
Nitrobenzene	1	1	1	Liquid, pungent odor; d 1.203 g/mL @ 20 °C; BP 211.0 °C; MP 5 °C; VP 37 kPa (0.28 mm Hg) @ 20 °C

Ca = potential human carcinogen

TABLE 2. ANALYTICAL MEASUREMENTS

Analyte	1 ppm = mg/m³ @ NTP	Range Studied (µg/sample)	LOD (µg/sample)	Precision ($\bar{S}_r$)
Aniline	3.87	31 to 255	4	0.016
o-Toluidine	4.46	30 to 252	3	0.015
Nitrobenzene	5.12	27 to 460	1	0.033

APPENDIX. PREPARATION OF SULFURIC ACID-TREATED FILTERS

Acid-treated Gelman Type A/E, 37-mm, glass fiber filters, or equivalent, are prepared by soaking each filter with 0.5 mL of 0.26 *N* sulfuric acid. (0.26 *N* sulfuric acid can be prepared by diluting 1.5 mL of 36 *N* sulfuric acid to 200 mL with deionized water.) The filters are dried in an oven at 100 °C for 1 h and then assembled into 37-mm polystyrene cassettes without support pads. The cassettes are sealed with shrink bands and the ends are plugged with plastic plugs.

Sulfuric acid-treated glass fiber filters also are available commercially—two treated filters preloaded in a 3-piece cassette. These samplers may be used, although this method specifies only one treated filter.