BENZIDINE and 3,3′-DICHLOROBENZIDINE

5509

(1) $(C_6H_4NH_2)_2$ MW: (1) 184.23 CAS: (1) 92-87-5 RTECS: (1) DC9625000
(2) $(C_6H_3ClNH_2)_2$ (2) 253.13 (2) 91-94-1 (2) DD0525000

METHOD: 5509, Issue 3 **EVALUATION:** FULL **Issue 1:** 15 May 1989
Issue 3: 29 February 2016

OSHA: lowest feasible; carcinogen (29 CFR 1910.1010)
NIOSH: lowest feasible; carcinogen (29 CFR 1910.1010)

PROPERTIES: (1) solid; MP 127 °C; BP 400 °C
(2) solid; MP 132 °C

SYNONYMS: (1) [1,1'-biphenyl]-4,4'-diamine; *p*-diaminodiphenyl and (2) 3,3′-Dichloro[1,1′-biphenyl]-4-4′-diamine

SAMPLING

SAMPLER: FILTER (13-mm glass fiber)

FLOW RATE: 0.2 L/min

VOL-MIN: 20 L @ 10 µg/m³
-MAX: 100 L

SHIPMENT: ship benzidine samples in dry ice

SAMPLE STABILITY: (1) 11 days @ 15 °C [1]
(2) 12 days @ 23 °C [1]

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: (1) 21 to 63 µg/m³ [1]
(2) 20 to 130 µg/m³ [1]

BIAS: (1) -3%
(2) -4.0%

OVERALL PRECISION ($\hat{S}_{rT}$): 0.07 [1]

ACCURACY: (1) ± 13.7%
(2) ± 15.2%

MEASUREMENT

TECHNIQUE: HPLC, UV DETECTION

ANALYTE: benzidine or 3,3'-dichlorobenzidine

DESORPTION: 0.5 mL 0.17% (v/v) trimethylamine in methanol; stand 60 min

INJECTION VOLUME: (1) 10 µL
(2) 15 µL

MOBILE PHASE: (1) 60% methanol/40% water
(2) 70% acetonitrile/30% water

FLOW RATE: 1.5 mL/min; ambient temperature

COLUMN: C18 US Pharmacopeia (USP) L1, 10 µm particles, 4-mm ID by 30-cm long

DETECTOR: UV @ 254 nm

CALIBRATION: solutions of analyte(s) in eluent

RANGE: 0.2 to 7 µg/sample

ESTIMATED LOD: 0.05 µg/sample [1]

PRECISION ($\bar{S}_r$): ≤0.07 [1]

APPLICABILITY: The working range for benzidine or 3,3'-dichlorobenzidine is 4 to 200 µg/m³ for a 50-L air sample. Benzidinium sulfate and 3,3'-dichlorobenzidine dihydrochloride will be collected and converted to benzidine and 3,3'-dichlorobenzidine, respectively, during sample preparation.

INTERFERENCES: Aniline interferes in the determination of benzidine but may be resolved [2]. 4,4'-Methylenebis(2-chloroaniline) interferes in the determination of 3,3'-dichlorobenzidine [1]. A number of compounds were shown not to interfere [1, 2] (see step 12, NOTE 2).

OTHER METHODS: This combines and replaces P&CAM 243 and P&CAM 246 [3].

REAGENTS:

1. Methanol, HPLC grade.
2. Acetonitrile, HPLC grade.
3. Triethylamine.
4. Water, distilled, deionized.
5. Benzidine.*
6. 3,3'-Dichlorobenzidine.*
7. Eluent: 0.17% (v/v) triethylamine in methanol. Dilute 170 µL triethylamine to 100 mL with methanol.
8. Calibration stock solution, 0.5 µg/µL. Dissolve 50 mg analyte in 100 mL eluent.
9. Recovery (R) stock solution, 0.5 µg/µL. Dissolve 50 mg analyte in 100 mL methanol.

*See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: 13-mm, Type AE, glass fiber filter in a 13-mm filter holder.
2. Personal sampling pump, 0.2 L/min, with flexible connecting tubing.
3. High-performance liquid chromatograph, UV detector, integrator and column (page 5509-1).
4. Test tubes, 1-mL, with polyethylene stoppers.
5. Syringes, glass, 10- and 25-µL, readable to 0.1 µL.
6. Pipets, delivery, 0.5- and 5-mL, graduated in 0.1 mL.
7. Flasks, volumetric, 10- and 100-mL.
8. Centrifuge.
9. Test tube shaker, vortex type.

SPECIAL PRECAUTIONS: Benzidine is a recognized human carcinogen and can be absorbed through the skin [4,5,6]. 3,3'-Dichlorobenzidine is a carcinogen [4,6]. Take appropriate precautions to avoid personal and area contamination.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Sample at 0.2 L/min for a total sample size of 20 to 100 L.
3. Cap the sampler.
4. Ship and store samples at -15 °C if benzidine may be present.
   NOTE: Samples may be stored at room temperature if only 3,3'-dichlorobenzidine is present.

SAMPLE PREPARATION:

5. Place glass fiber filter in a test tube.
6. Add 0.5 mL eluent to each test tube. Seal each test tube and shake them on a test tube shaker.
7. Allow samples to stand for 1 h with intermittent shaking.
8. Centrifuge each sample for 10 min.

CALIBRATION AND QUALITY CONTROL:

9. Calibrate daily with at least six working standards over the range 0.05 to 7 µg analyte per sample.
   a. Deliver aliquots of calibration stock solution with microliter syringe to eluent in 10-mL volumetric flasks and dilute to the mark.
   b. Analyze together with the samples and blanks (steps 12 through 14).
   c. Prepare calibration graph (peak area vs. µg analyte).
10. Determine recovery (R) at least once per year for each lot of filters. Prepare four filters at each of five levels, plus three media blank filters.
    a. Place sample filters into separate test tubes.
    b. Inject an aliquot of R stock solution, or a dilution thereof in methanol, directly onto the filter.
    c. Cap the test tubes. Allow to stand overnight.
    d. Prepare (steps 5 through 8) and analyze (steps 12 through 14) with working standards.
    e. Prepare a graph of R for each filter vs. µg analyte recovered.

11. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration and R graphs are in control.

MEASUREMENT:

12. Set the liquid chromatograph to conditions given on page 5509-1 for analyte of interest.
    NOTE 1: If aniline is present, use a C18 column and follow the procedures found in reference [2].
    NOTE 2: The following compounds were found not to interfere with the determination of either compound: *o*-, *p*- and *m*-chloroaniline; 4,4'-methylenedianiline and ß-naphthylamine. 2-Chloro-4-methylaniline; 3,3'-dichlorobenzidine; 4,4'-methylenebis(2-chloroaniline); hydrazobenzene; and 1,2- and 1,4-naphthoquinone do not interfere in the determination of benzidine. Benzidine, aniline, *N*-methylaniline, 2-toluidine and 3,3'-dimethylbenzidine will not interfere in the determination of 3,3'-dichlorobenzidine [1].
13. Inject an aliquot (see page 5509-1 for appropriate size).
14. Measure peak area.

CALCULATIONS:

15. Read the mass, µg (corrected for R), of analyte found on the sample filter (W) and on the media blank filter (B) from the calibration graph.
16. Calculate concentration, C, of analyte in the air volume sampled, V (L):

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

EVALUATION OF METHOD:

This method was evaluated over the range 21 to 63 µg/m^3 for benzidine and the range 20 to 130 µg/m^3 for 3,3'-dichlorobenzidine. The generated atmospheres for both compounds were at 30 °C and 80% relative humidity. The sampling rate was 0.8 L/min. The pooled overall precision ($\hat{S}_{rT}$) was 0.07 for 29 benzidine samples and 0.07 for 28 3,3'-dichlorobenzidine samples. The sampling methods were evaluated for effects of temperature (25, 30, and 35 °C) and relative humidity (20 and 80%). No detectable quantities of either benzidine or 3,3'-dichlorobenzidine were found on backup silica gel tubes [1]. At 180 °C, vapors of these carried by a stream of dry nitrogen at 0.3 L/min did not break through the backup silica gel in 3 h [1].

The average recovery of benzidine from filters was determined to be 97% over the range 0.2 to 2.0 µg when stored at -15 °C for 11 days. Recoveries dropped to 89% and 75% after 15 days and 21 days, respectively. Recoveries of benzidine and benzidinium sulfate from filters and silica gel indicated that the compounds were unstable in these matrices at ambient temperature. Recoveries over the range of 0.5 to 5 µg 3,3'-dichlorobenzidine and its dihydrochloride were 96% after 21 days from both filters and silica gel stored at -15 °C and at ambient temperature [1].

REFERENCES:

[1] Morales R, Rappaport SM, Weeks, Jr. RW, Campbell EE, Ettinger HJ [1977]. Development of sampling and analytical methods for carcinogens, January 1 - September 30, 1976, Los Alamos Scientific Laboratory, Los Alamos, NM. Available as LA-7058-PR, from NTIS. Unpublished.

[2] Kennedy ER, Seymour MJ [1981]. Development of an analytical method for benzidine-based dyes. In: Chemical hazards in the Workplace, ACS symposium series: Vol. 149. Washington, DC: American Chemical Society, pp 21-35.

[3] NIOSH [1977] P&CAM 243 and P&CAM 246. In: Taylor DG, ed. NIOSH manual of analytical methods, 2nd ed. (Vol. 1) Cincinnati, OH: U.S. Department of Health, Education, and Welfare, Centers for Disease Control, National institute for Occupational Safety and Health, DHEW (NIOSH) Publication No. 77-157-B.

[4] NIOSH [1980]. NIOSH technical report: carcinogenicity and metabolism of azo dyes, especially those derived from benzidine. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health.

[5] NIOSH/NCI [1978]. Current intelligence bulletin 24: Direct Blue 6, Direct Black 38, and Direct Brown 95 benzidine-derived dyes. Cincinnati, OH: U.S. Department of Health, Education, and Welfare, Center for Disease Control, National Institute for Occupational Safety and Health, DHEW (NIOSH) Publication No. 78-148.

[6] NIOSH [1981]. Occupational health guidelines for chemical hazards. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 81-123.

METHOD REVISED BY:

M. J. Seymour, NIOSH.