DYES, BENZIDINE-, o-TOLIDINE-, o-DIANISIDINE- 5013

Table 1 MW: Table 1 CAS: Table 1 RTECS: Table 1

METHOD: 5013, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 May 1989**
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OSHA : no PEL
NIOSH: lowest feasible; carcinogen
ACGIH: carcinogen

PROPERTIES: colored powders; water soluble; VP not significant

SYNONYMS: Table 1.

SAMPLING

SAMPLER:	FILTER (5-µm PTFE membrane)
FLOW RATE:	1 to 3 L/min
VOL-MIN:	150 L @ 0.1 mg /m^3
-MAX:	500 L
SHIPMENT:	keep samples dry and cool; protect from light
SAMPLE STABILITY:	at least 7 days @ 25 °C in the dark
FIELD BLANKS:	2 to 10 field blanks per set
BULK SAMPLE:	include 1 bulk sample for each dye on the filter

ACCURACY

RANGE STUDIED:	not studied
BIAS:	not identified
OVERALL PRECISION ($\hat{S}_{rT}$):	not evaluated
ACCURACY:	not determined

MEASUREMENT

TECHNIQUE:	HPLC, UV DETECTION
ANALYTE:	benzidine, o-tolidine, o-dianisidine
DESORPTION:	2 mL H_2o; utrasonic
REACTION:	reductive cleavage of dye to free amine with sodium hydrosulfite
INJECTION VOLUME:	10 µL
MOBILE PHASE:	60% methanol/ 40% phosphate buffer; ambient temperature
COLUMN:	10 cm x 8-mm ID, Waters Radial Pak C_{18}, 10 µm particles, with Radial Compression Module of equivalent
DETECTOR:	UV detector @ 280 nm
CALIBRATION:	solutions of benzidine, o-tolidine and o-dianisidine in methanol
RANGE:	ca. 15 to 250 µg per sample (Table 1)
ESTIMATED LOD:	3 µg benzidine per sample [1]
PRECISION ($\bar{S}_r$):	0.04 to 0.08 [2,3]

APPLICABILITY: The working range is ca. 0.06 to 8 mg/m^3 for a 250-L air sample. This method determines benzidine, o-tolidine or o-dianisidine from dyes based on these amines, but will not distinguish different dyes based on the same amine. The method will not distinguish benzidine obtained from reduction of the dyes and free benzidine in the dye formulation.

INTERFERENCES: Aniline, azobenzene, p-aminophenol, p-phenylenediamine or p-nitroaniline do not interfere in the measurement when present in equimolar amounts.

OTHER METHODS: This revises P&CAM 325 [2,3]. P&CAM 234 [4,5], a general colorimetric method for diazonium salt and azo dyes, has not been revised

REAGENTS:

1. Water, deionized and distilled.
2. Methanol, HPLC Grade.
3. Benzidine.*
4. o-Tolidine.*
5. o-Dianisidine.*
6. Calibration stock solutions. Dilute the following amounts to 10 mL with methanol. Stable one month at 4 °C.
   a. Benzidine, 15.6 mg
   b. o-Tolidine, 15.3 mg
   c. o-Dianisidine, 5.6 mg
7. Disodium hydrogen phosphate, Na_2HPO_4.
8. Potassium dihydrogen phosphate, KH_2PO_4.
9. Sodium hydrosulfite, $Na_2S_2O_4$.
10. HPLC mobile phase buffer. Dilute 3.39 g KH_2PO_4 and 4.30 g Na_2HPO_4 to 1 L with water. Prepare daily.
11. Reduction buffer.
    a. Solution A. Dilute 1.179 g KH_2PO_4 and 4.30 g Na_2HPO_4 to 1 L with water. Prepare Daily.
    b. Solution B. Dilute 11.79 g KH_2PO_4 and 43.00 g Na_2HPO_4 to 1 L with water. Prepare daily.
12. Reducing solution. Dilute 200 mg $Na_2S_20_4$ to 10 mL with the appropriate reduction buffer of Solution A or B (see Table 1) immediately before use.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: PTFE filter, 5-µm, 37-mm (Millipore Mitex or equivalent), with backup pad in a three-piece plastic cassette filter holder.
2. Personal sampling pump, 1 to 3 L/min, with flexible connecting tubing.
3. High pressure liquid chromatograph eqipped with 280-nm UV detector, integrator and column (page 5013-1), Waters Model RCM 100 Radial Compression Module with Waters Radial Pak C_{18} column or equivalent.
4. Syringe or autosampler for HPLC injection.
5. Syringes, volumetric, 10- and 25- and 50 µL.
6. Flasks, volumetric, 10- and 100-mL, 1-L.
7. Vials, 4-mL, with screw caps.
8. Pipets, volumetric, 1-mL.
9. Tweezers.
10. Beakers, 50-mL.
11. Ultrasonic water bath.

SPECIAL PRECAUTIONS: NIOSH recommends that benzidine-and benzidine-congener-based dyes be recognized and handled as human carcinogens because they can be reduced to the free aromatic amine in living systems and eliminated by the usual metabolic pathways [4,6],

Benzidine is a recognized human carcinogen and regulated by the Occupational Safety and Health Administation. o-Tolidine and o-dianisidine are currently suspect carcinogens and should be handled accordingly [4,6].

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Sample at an accurately known flow rate between 1 and 3 L/min for a total sample size of 150 to 500 L.

SAMPLE PREPARATION:

3. Remove the filter from the cassette with clean tweezers and place it face up in a 50-mL beaker.
4. Add 1.0 mL H_2O to the beaker and swirl to wet the entire deposit.
5. Add another 1.0 mL H_2O to the beaker and swirl.
6. Turn the filter sample side down and place the beaker in an ultrasonic bath for 15 min.
7. Pipet 1.0 mL desorbed sample solution to a 4-mL vial.
8. Add 1.0 mL freshly prepared reducing solution.
   NOTE: Where the composition of the sample dye is unknown or different from the dyes listed in Table 1, reduce a 1-mL aliquot of the desorbed dye solution with the reducing solution made from buffer A. If precipitation of the dye from solution is observed at this point, reduce the remaining 1-mL aliquot of the desorbed dye solution with the reducing solution made with buffer B.
9. Cap the vial and allow to stand with occasional shaking for at least 1 h or until the change in solution color is completed.

CALIBRATION AND QUALITY CONTROL:

10. Calibrate daily.
    a. Dilute aliquots of the calibration stock solutions for each of the amines (benzidine, o-tolidine, o-dianisidine) to 10 mL with methanol in volumetric flasks. Prepare at least six working standards to cover the ranges of interest (0.38 to 16 µg/mL benzidine; 0.77 to 15.3 µg/mL o-tolidine; 0.56 to 11.7 µg/mL o-dianisidine).
    b. Analyze the working standards together with samples and blanks (steps 12 through 14).
    c. Prepare separate calibration graphs (peak area vs. µg per sample) for benzidine, o-tolidine. and o-dianisidine. At the completion of step 9, the total effective volume of the solution containing the entire sample is 4 mL.
11. Determine recovery if the filter sample is known to contain only one dye.
    a. Prepare a solution of a known concentration of the bulk sample of the dye in 10 mL H_2O.
    b. Add known volumes of this solution to PTFE filters using a microliter syringe and allow to dry. Prepare three filters at each of five concentration levels.
    c. Analyze the filters by steps 3 through 9 and 12 through 14.
    d. On the day of analysis of the filters, add the same volume of dye solution which was spiked onto the filters to 2.0 mL H_2O. Reduce this solution (steps 7 through 9) and analyze together with samples and standards (steps 12 through 14).
    e. Calculate recovery (µg benzidine, o-tolidine, or o-dianisidine recovered from the filter/µg from step 11.d).
    f. Prepare graph of recovery vs. µg benzidine, o-tolidine or o-dianisidine recovered.

MEASUREMENT:

12. Set the HPLC to conditions given on page 5013-1 and in Table 1.
13. Inject 10 µL sample into the HPLC. Make duplicate injections of samples and standards.
14. Measure peak areas.

CALCULATIONS:

15. Read the mass, µg (corrected for recovery, if applicable) of the free amine corresponding to each peak area for the sample (W) and average media blank (B) from the appropriate calibration graph.
16. Calculate the concentration, C, of benzidine, o-tolidine or o-dianisidine in the air volume sampled, V (L):

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

EVALUATION OF METHOD:

This method was laboratory evaluated over the ranges listed in Table 1 [2]. The concentration of dye remaining after reduction varied from 0 to 6% as determined by visible spectrophotometry. The reduced free amine was confirmed by GC/MS. The lower end of the measurement range was defined as the level which gave at least 75% recovery with approximately 10% $\bar{s}_r$. For C. I. Direct Red 28, the range covered 27.3 to 273 µg dye per sample. The lower level of this range gave 94.4% recovery ($\bar{s}_r$ = 6.3%) based on free benzidine for six replicate samples. For the other three dyes the results were as follows: C. I. Direct Blue 6, 15.0 to 300.0 µg dye per sample, 100.3% recovery ($\bar{s}_r$ = 4.34%); C. I. Direct Brown 95, 24.3 to 243 µg dye per sample, 78.5% recovery ($\bar{s}_r$ = 6.7%); C. I. Direct Black 38, 12.5 to 250.0 µg dye per sample, 78.1% recovery ($\bar{s}_r$ = 12.8%). The pooled precisions ($\bar{s}_r$) for samples at three concentrations in the measurement range for each dye are as follows: Direct Red 28, 0.045; Direct Blue 6, 0.061; Direct Brown 95, 0.072; Direct Black 38, 0.078. Since the method does not distinguish between various benzidine-based dyes, recovery correction factors cannot be applied unless a single known dye is contained on the filter. Recovery studies must be performed on the bulks collected with the samples because of variability in sample purity and variety of benzidine-based dyes. Recovery studies on spiked samples stored at 75% relative humidity indicated no change due to humidity in the recoveries or sample stability.

A user check of this method [1] gave estimated LODs for benzidine (3 µg per sample; 0.7 µg/mL) and dianisidine (4.5 µg per sample; 1.1 µg/mL). The LODs could be lowered to 0.06 µg/mL for benzidine and 0.07 µg/mL for dianisidine by using fluorescent detection with excitation at 285 nm and emission at 375 nm.

REFERENCES:

[1] User check, Wisconsin Occupational Health Laboratory (NIOSH, unpublished, September 27, 1984).
[2] NIOSH Manual of Analytical Methods, 2nd ed., Vol. 6, P&CAM 325, U.S. Department of Health and Human Services, Publ. (NIOSH) 80-125 (1980).
[3] Kennedy, Eugene R. and Martha J. Seymour. Development of an Analytical Method for Benzidine-Based Dyes, Chemical Hazards in the Workplace Measurement and Control, ACS Symposium Series 149, American Chemical Society, Washington, DC, 21-35 (1981).
[4] Special Occupational Hazard Review for Benzidine-Based Dyes, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 80-109 (1979).
[5] NIOSH Manual of Analytical Methods, 2nd ed., Vol. 1, P&CAM 234, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-A (1977).
[6] Preventing Health Hazards from Exposure to Benzidine Congener Dyes, U.S. Department of Health and Human Services, Publ. (NIOSH) 83-105 (1983).

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. . TABLE 1. BENZIDINE- AND BENZIDINE-CONGENER-BASED DYES.

Color Index Name	C.I. Number	Molecular Formula	M.W.	Synonyms [4]	Range[a] µg per sampler	Range[a] µg/m^3	HPLC Flow rate (mL/min)	Reduction Buffer[b]
BENZIDINE-BASED: C.I. Direct Red 28	22120	$C_{32}H_{22}N_6S_2O_6Na_2$	696.67	Congo Red; CAS #573-58-0 RTECS QK1400000	27.3-273	54.6-546	2	B
C.I. Direct Blue 6	22610	$C_{32}H_{20}N_6S_4O_{14}Na_4$	932	Amidine Blue 2B; CAS #2602-46-2 RTECS QJ6400000	15.0-300	30.0-600	2	B
C.I. Direct Brown 95	30145	$C_{31}H_{18}N_6O_9Na_2Cu$	759	Chrome Leather Brown; CAS #16071-86-6 RTECS GL7375000	24.3-242	48.6-485	2	B
C.I. Direct Black 38	30235	$C_{34}H_{25}N_9S_2O_7Na_2$	781	Amidine Black GA; CAS #1937-37-7 RTECS QJ6160000	12.5-250	25.0-500	1	A
o-TOLIDINE-BASED: C.I. Direct Red 2	23500	$C_{34}H_{26}N_6S_2O_6Na_2$	724.74	Benzopurpurine 4B; CAS #992-59-6 RTECS QK1765000	16.7-334	33.4-668	1	A
o-DIANISIDINE-BASED: C.I. Direct Blue 8	24140	$C_{34}H_{24}N_4S_2O_{10}Na_2$	758.69	Direct Azurine G; Benzo Azurine G; CAS #2429-71-2	10.2-204	20.4-408	1	A

[a] Air concentrations calculated from analytical results with an air volume of 500 L assumed.
[b] The letters "A" and "B" refer to the reduction buffer solutions described in the REAGENTS section, items 11.a and b.