BENZIDINE in urine

$C_{12}H_{12}N_2$ MW: 184.24 CAS: 92-87-5 RTECS: DC9625000

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BIOLOGICAL INDICATOR OF: exposures to benzidine and benzidine-based azo dyes.

SYNONYMS: [1,1'-biphenyl]-4,4'-diamine

BIOLOGICAL SAMPLING		MEASUREMENT	
SPECIMEN:	urine, 2 to 3 h after exposure	**TECHNIQUE:**	GAS CHROMATOGRAPHY, ECD
VOLUME:	100 mL in polyethylene bottle	**ANALYTE:**	N,N'-diheptafluorobutyryl benzidine
PRESERVATIVE:	2 drops 12 N HCl after collection	**INJECTION VOLUME:**	3 µL, Grob type-splitless
SHIPMENT:	frozen and shipped in dry ice	**COLUMN:**	15 m × 0.25-mm I.D. capillary; WCOT-SE-54
SAMPLE STABILITY:	unknown	**CARRIER GAS:**	5 % methane in argon, 1 mL/min
CONTROLS:	at least 3 urine specimens from non-exposed individuals per study; 1 per 10 samples	**TEMPERATURE-INJECTOR:**	260°C
		-DETECTOR:	350°C
		-COLUMN:	120°C for 2 min; 40°/min to 280°; hold 6 min
		CALIBRATION:	solutions of benzidine in benzene with internal standard
		QUALITY CONTROL:	spiked urine corrected for creatine content
		RANGE:	10 to 1000 µg/L
		RECOVERY:	92%
		ESTIMATED LOD:	5 µg/L
		PRECISION ($\hat{S}_r$):	0.11
		ACCURACY:	± 29%

APPLICABILITY: This procedure is useful for monitoring exposures to benzidine or benzidine-based azo dyes which are absorbed via the skin, lung, or gastrointestinal tract, and whose metabolites are benzidine or acetylbenzidine. This procedure qu antitates benzidine, N-acetylbenzidine, N,N'-diacetylbenzidine, as conjugates of benzidine. Maximum urinary levels occur 2 to 3 h after exposure [1].

INTERFERENCES: None known.

OTHER METHODS: This method is based on references [2] and [3].

REAGENTS:

1. Hydrochloric acid, conc. (sp. gr. 1.18).
2. Sodium hydroxide, 10 N. Dissolve 400 g NaOH in 1:1 methanol:water (v/v) to make 1 L solution.
   CAUTION: EXOTHERMIC REACTION.
3. Sodium hydroxide, 5 N. Dilute 10 N with 1:1 methanol:water
4. Acetone, hexane, methanol, and benzene*, pesticide grade.
5. Hexane:benzene, 3:2 (v/v).
6. Sodium sulfate (anhydrous) extracted in Soxhlet extractor for 24 h with benzene.
7. Trimethylamine stock solution.
   a. Dissolve 2 g $(CH_3)_3NHCl$ in 5 mL 5 N NaOH.
      CAUTION: EXOTHERMIC REACTION
   b. Extract four times with 5-mL portions of benzene.
   c. Percolate each 5 mL extract through a 2-cm anhydrous sodium sulfate column.
   d. Keep in the dark and refrigerated when not in use. Reagent stability unknown.
8. Trimethylamine working solution.
   Dilute trimethylamine stock solution 1:20 with benzene. Reagent stability unknown.
9. Benzidine stock solution, 100 µg/mL.* Dissolve 10 mg benzidine, accurately weighed, in benzene to make 100 mL solution. Refrigerate. Discard if a color develops.
10. Methylene dianiline stock solution, 100 µg/mL. Dissolve 10 mg methylenedianiline,, accurately weighed, in benzene to make 100 mL solution. Refrigerate. Discard if color develops.
11. Derivatized methylenedianiline stock solution, 1 µg/mL.
    a. Add 0.1 mL methylenedianiline stock solution to 1.5 mL benzene and 0.5 mL trimethylamine working solution.
    b. Derivatize and wash (steps 12 and 13).
    c. Percolate the solution through 2 cm anhydrous sodium sulfate.
    d. Dilute to 10.0 mL with benzene. Stable at least one month.
12. Florisil, PR grade, 60/100 mesh; activated at 650°C.
    a. Extract 24 h in a Soxhlet extractor with benzene.
    b. Heat to 130°C for 24 h.
    c. Cool and add 10 g distilled water/100g Florisil. Shake overnight.

EQUIPMENT:

1. Bottles, polyethylene, 125-mL.
2. Gas chromatograph with $_{28}Ni^{63}$ electron capture detector, temperature programming, capillary column option, Grob-type injection system, and electronic integrator.
3. Shaker and rotator.
4. Evaporator and vortex mixer.
5. Centrifuge (refrigerated optional).
6. Waterbath with a temperature range 30 to 100°C.
7. Soxhlet extractor.
8. Culture tubes, 25 × 200-mm, 20 × 125-mm, and 16 × 150-mm, with PTFE-lined screw caps.**
9. Pipettes, 1-, 5- and 10-mL and Pasteur (disposable).**
10. Graduated centrifuge tubes, 15-mL, glass-stoppered.**
11. Volumetric flasks.**

** Wash with detergent, clean with chromic acid, rinse with distilled water, acetone, and benzene.

13. Buffer. Dissolve 136 g KH_2PO_4 in 900 mL H_20. Adjust to pH 6 with 10 N NaOH. Dilute to 1 L with H_20 and extract once with 100 mL benzene.
14. Paraffin oil.
15. Heptafluorobutyric anhydride.
16. Nitrogen gas, compressed, purified.

* See SPECIAL PRECAUTIONS.

SPECIAL PRECAUTIONS: Samples of urine collected from humans pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this is much less. Those who handle urine specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided. Benzidine and benzene are documented human carcinogens and must be handled in compliance with 29 CFR 1910.1005 and 1910.1028.

SAMPLING:

1. Collect a spot urine sample in a polyethylene bottle. Add 2 drops 12 N HCl as a preservative.
2. Freeze the samples and ship in an insulated container with dry ice.
3. Keep the samples frozen until analysis.

SAMPLE PREPARATION:

4. Perform a creatinine determination on an aliquot of the sample (e.g., [4]).
5. Pipet 10 mL of the urine specimen into a 50-mL culture tube containing 10 mL 10 N NaOH.
6. Seal the tube and incubate 2 h at 80 °C.
7. Cool to room temperature, add 20 mL benzene, seal, and shake 1 h.
8. Remove the benzene layer and percolate it through a 2-cm column of anhydrous sodium sulfate. Collect the eluate in a 25 × 200-mm culture tube.
9. Repeat step 8 twice. Combine the extracts.
10. Add three or four drops of paraffin oil to the extracts and concentrate the extracts to near dryness under a gentle stream of nitrogen.
11. Add 1.5 mL benzene and 0.5 mL trimethylamine working solution.
12. Add 50 µL heptafluorobutyric anhydride. Seal and place in a 50 °C waterbath for 20 min. Cool to room temperature.
13. Wash the derivatized extract.
    a. Add 2 mL pH 6 buffer solution. Seal and shake 2 min.
    b. Centrifuge 2 min. Discard the bottom (aqueous) layer.
    c. Repeat the washing with two additional 2-mL portions of pH 6 buffer.
14. Place a small glass wool plug in a chromatographic column and add 1.6 g Florisil. Top with 2 cm anhydrous sodium sulfate. Wash the column with 50 mL hexane.
15. Add the buffer-washed, derivatized extract to the Florisil column. Rinse the culture tube twice with ca. 2 mL benzene and add the rinsings to the Florisil column.
16. When the top of the extract is in the sodium sulfate layer, add 10 mL 3:2 (v/v) hexane:benzene. Discard the eluate.
17. Elute the N,N'-diheptafluorobutyryl benzidine with 15 mL benzene.
18. Add 0.5 mL derivatized methylenedianiline stock solution to the eluate. Concentrate to 10 mL

under a gentle stream of nitrogen.

CALIBRATION AND QUALITY CONTROL:

19. Prepare six working standards containing between 0 and 10 µg benzidine.
    a. Add aliquots of benzidine stock solution to 1.5 mL benzene and 0.5 mL trimethylamine working solution.
    b. Perform steps 12 and 13.
    c. Percolate the benzene layer through 2 cm anhydrous sodium sulfate.
    d. Add 0.5 mL derivatized methylenedianiline stock solution.
    e. Dilute to 10.0 mL with benzene.
    f. Analyze the working standards together with samples and blanks (step 21 and 22).
    g. Prepare calibration graph (concentration of standards vs. ratio of peak areas of benzidine to methylenedianiline).
20. Analyze a spiked urine for every 10 samples (minimum three per study).

MEASUREMENT:

21. Set the chromatograph according to manufacturer's recommendations and conditions given on page 8306-1.
22. Inject 3 µL derivatized sample extract (step 18). Measure peak areas of both methylenedianiline and benzidine. Calculate the ratio of peak areas (benzidine to methylenedianiline). The t_r of methylenedianiline is 7.6 min while the t_r of benzidine is 8.0 min under these conditions.

CALCULATIONS:

23. Determine benzidine urine concentrations (µg/mL) by comparing the ratio of benzidine methylenedianiline peak areas for the samples to those obtained for standards on the calibration graph. Report the results as µg benzidine/g creatinine.

GUIDES FOR INTERPRETATION:

Benzidine is an OSHA-regulated human carcinogen. Although no specific standard has been set, the fact that benzidine is a human carcinogen means it or its metabolites should not be detected in urine or any other physiological fluid. However, a level of 0.010 µg/mL has been suggested as an indicator of excessive exposure [1].

REFERENCES:

[1] Baselt, R. C. Biological Monitoring Methods for Industrial Chemicals, 43, Biomedical Publications, Davis, CA (1980).
[2] Nony, C. R., and M. C. Bowman. Trace Analysis of Potentially Carcinogenic Metabolites of an Azo Dye and Pigment in Hamster and Human Urine as Determined by Two Chromatographic Procedures, J. Chromatographic Sci., 18, 64 (February, 1980).
[3] Benzidine-Based Dyes, 46, U.S. Department of Health and Human Services, Publ. (NIOSH) 80-109 (1980).
[4] Tietz, N. W. Fundamentals of Clinical Chemistry, 2nd ed., 994-997, W. B. Saunders Co., Philadelphia, PA (1976).

METHOD REVISED BY:

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