

PENTACHLOROPHENOL in urine

8303

C₆Cl₅OH

MW: 266.33

CAS:87-86-5

RTECS: SM6300000

METHOD: 8303

EVALUATION: FULL

Issue 1: 15 May 1989

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BIOLOGICAL INDICATOR OF: exposure to pentachlorophenol.

SYNONYMS: PCP; Penta.

SAMPLING	MEASUREMENT
<p>SPECIMAN: urine end of shift, mid to late in work week</p> <p>VOLUME: 100 mL in polyethylene bottle</p> <p>PRESERVATIVE: 2 to 3 drops concentrated HCl added after collection</p> <p>SHIPMENT: ship frozen in dry ice</p> <p>SAMPLE STABILITY: 40 days if kept frozen</p> <p>CONTROLS: collect 3 urine specimens from unexposed workers per study or 1 control urine per 10 samples</p>	<p>TECHNIQUE: GAS CHROMATOGRAPHY, ECD</p> <p>ANALYTE: pentachloroanisole (PCP methyl ether)</p> <p>INJECTION VOLUME: 5 µL</p> <p>COLUMN: 1.8 m x 4-mm ID glass, 4% SE-30, 6% OV-210 on 80/100 mesh silanized support</p> <p>CARRIER GAS: 5% methane in argon, 60 mL/min</p> <p>TEMPERATURE-INJECTOR: 220 °C</p> <p>-COLUMN: 190 °C</p> <p>-MANIFOLD: 250 °C</p> <p>-DETECTOR: 300 °C</p> <p>CALIBRATION: solutions of pentachloroanisole in hexane</p> <p>QUALITY CONTROL: spike urine pools; corrected for creatinine content</p> <p>RANGE: 5 to 1000 µg/L</p> <p>ESTIMATED LOD: 1 µg/L [1]</p> <p>RECOVERY: 0.947</p> <p>PRECISION (S_r): 0.03 [1]</p> <p>ACCURACY: ± 10.3%</p>

APPLICABILITY: This method measures free and conjugated PCP after hydrolysis and is useful when monitoring chronic PCP exposure via skin contact, ingestion or inhalation. Maximum urine levels are found 42 h after exposure [2]. Acute exposures are best monitored using Method 8001 (Pentachlorophenol in blood). Urine contains about 82% free PCP and 13% PCP glucuronide [1].

INTERFERENCES: Numerous potential interferences that can be extracted from urine include chloronaphthylenes, polychlorinated biphenyls and Diuron; these are eliminated by sample cleanup.

OTHER METHODS: This method combines and replaces P&CAM 230 [3] and P&CAM 358 [1].

REAGENTS:

1. Hexane, acetone, and benzene* (pesticide grade).
2. 20% benzene in hexane (v/v).
3. 10% benzene in hexane (v/v).
4. Hydrochloric acid (conc.).
5. Sodium bisulfite.
6. Sodium sulfate.
7. Alumina (acid-washed), Brockman Activity I, 80/200 mesh.
8. Pentachloroanisole (available from EPA Analytical Reference Lab).
9. Calibration stock solution equivalent to 100 mg/L PCP. Weigh 0.0105 g pentachloroanisole; dilute to 100 mL in a volumetric flask with hexane. Stable 2 months if stored in refrigerator.
10. Diazomethane*. Prepared from Diazald kit according to manufacturer's instructions [4].
11. 5% methane in argon.

* See Special Precautions.

EQUIPMENT:

1. Bottles, polyethylene, 125-mL, screw cap.**
2. Gas chromatograph with ⁶³Ni electron capture detector.
3. Culture tubes, 16 x 150-mm and 16 x 125-mm, with PTFE-lined screw caps.**
4. Mixer, rotary, variable speed.
5. Centrifuge (refrigerated, optional).
6. Chromatography column, 7-mm ID x 200 mm, with 50-mL reservoir and PTFE stopcock.**
7. Syringes, 10- μ L, glass.
8. Pipets, 1000-, 500-, 250-, 100-, 50-, 25- and 10- μ L.**
9. Flasks, volumetric, 10- and 100-mL.**
10. Pipets, Pasteur, disposable.
11. Tubes, centrifuge, graduated, glass-stoppered, 15-mL.**
12. Waterbath, 100 °C.

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

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. Benzene is a documented human carcinogen and must be handled in compliance with 29 CFR 1910.1005.

Diazomethane and its precursor, N-methyl-N-nitroso-p-toluene sulfonamide are potent mutagens. Handle the bulk precursor in a glove box. Generate diazomethane behind an explosion shield in a hood.

SAMPLING:

1. Collect a spot urine sample of 100 mL in a 125-mL polyethylene bottle.
2. Add two or three drops conc. HCl to the sample as a preservative.
3. Freeze the samples and pack them in an insulated shipper (e.g., styrofoam with dry ice).

SAMPLE PREPARATION:

4. Perform a creatinine determination on an aliquot of the urine (e.g., [5]).
5. Transfer a 4-mL aliquot of each urine sample to a 16 x 150-mm culture tube.
6. Add 1.0 mL conc. HCl and 100 mg sodium bisulfite to each culture tube.
7. Cap and place the culture tubes in a boiling waterbath for 1 h. Shake gently at 15-min intervals.
8. Cool the culture tubes to room temperature.
9. Add 5 mL benzene to each culture tube. Extract on a rotary mixer at 60 rpm for 1 h.
10. Centrifuge at 3000 rpm. Transfer benzene layer to a 16 x 125-mm culture tube. Repeat the extraction with an additional 5 mL benzene. Combine extracts.
11. Concentrate the extract to approximately 0.6 mL under a gentle stream of nitrogen.

12. Add 10 mL of diazomethane reagent (use hood). Let stand under hood for 1 h.
13. Concentrate under a hood to 0.6 mL under a gentle stream of nitrogen.
14. Add 4 mL hexane. Evaporate the solution to approximately 0.6 mL.
15. Add 4 g acid-washed alumina to a 7-mm ID x 200 mm chromatography column. Cap with 1.6 g anhydrous sodium sulfate. Rinse the column with 20% benzene in hexane. Air-dry. Place the column in a 130 °C oven overnight. Cool the column to room temperature before use.
16. Wet the column with 5 mL hexane (use fume hood).
17. Add the concentrated derivatized extract when the solvent layer reaches the top of the sodium sulfate layer.
18. Rinse the culture tube three times with 0.5 mL hexane. Add the rinsings to the column.
19. Add 3.5 mL hexane to the column. Discard the hexane eluate.
20. Elute pentachloroanisole, the PCP derivative, with 20 mL 10% benzene in hexane.
21. Concentrate the sample to 2.0 mL in a graduated centrifuge tube.

CALIBRATION AND QUALITY CONTROL:

22. Prepare at least six working standards in the range 5 to 1000 µg/L PCP by dilution of the calibration stock solution with hexane.
23. Prepare a calibration graph (peak area of analyte vs. µg/L PCP).
24. Maintain standardization by injecting a working standard after each five sample injections.
25. Run a spiked urine every ten samples or if less than ten determinations are required, run at least three spiked urines per run or study.
NOTE: Urine used for spikes must be analyzed before use to determine background levels.

MEASUREMENT:

26. Set the gas chromatograph according to manufacturer's instructions and to conditions on page 8303-1.
27. Inject 5-µL sample extract from step 21. Measure peak area of analyte.
NOTE: The retention time for pentachloroanisole is about 4.7 to 5.0 min.

CALCULATIONS:

28. Use the analyte peak area from the samples and the calibration graph to determine the PCP concentration in the extract analyzed, C_e (µg/L PCP).
29. Calculate the urine PCP concentration, C (µg/L PCP), by applying the extract concentration factor 2 (4 mL urine yields 2 mL extract):

$$C = 0.5C_e, \mu\text{g/L PCP.}$$

30. Express PCP concentration as µg PCP/g creatinine.

GUIDES TO INTERPRETATION:

Urine pentachlorophenol levels for unexposed populations are reported to be between 20 and 40 µg/L PCP [6,7,8]. Symptoms have been reported at 200 µg/L PCP and levels in excess of 20 mg/L are fatal [7,9]. Lauwerys suggests an upper limit of 1 mg/g creatine [10]. The ACGIH Biological Exposure Index is 2 mg PCP/g creatinine [11].

EVALUATION OF METHOD:

The recovery of PCP from spiked urine samples was 93 to 96% for specimens containing 5 to 1000 µg PCP/L urine. The recovery of PCP from frozen urine samples spiked with 100 ng PCP/L urine and stored up to 36 days ranged from 85 to 91%.

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METHOD REVISED BY:

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