| | | | |
|---|---|---|---|
| (1) $C_6H_5OH$ | MW: 94.11 | CAS: 108-95-2 | RTECS: SJ3325000 |
| (2) $CH_3C_6H_4OH$ | MW: 108.14 | CAS: 106-44-5 | RTECS: GO6475000 |

**METHOD: 8305, Issue 2** **EVALUATION: PARTIAL** **Issue 1: 15 May 1985**
**Issue 2: 15 August 1994**

**BIOLOGICAL INDICATOR OF:** exposure to phenol, benzene, and p-cresol.

**SYNONYMS:** (1) phenol: carbolic acid
(2) p-cresol: 4-methylphenol

| SAMPLING | | MEASUREMENT | |
|---|---|---|---|
| **SPECIMEN:** | two spot urine samples (before and after exposure) | **METHOD:** | GAS CHROMATOGRAPHY, FID |
| **VOLUME:** | 50 to 100 mL in polyethylene screw-cap bottle containing preservative | **ANALYTE:** | phenol and p-cresol |
| **PRESERVATIVE:** | few crystals of thymol | **TREATMENT:** | acid hydrolysis; extraction |
| **SHIPMENT:** | freeze urine; ship in dry ice in an insulated container | **INJECTION VOLUME:** | 5 µL |
| **SAMPLE STABILITY:** | stable for 4 days @ 25 °C and for 3 months @ -4 °C | **TEMPERATURE-INJECTION:**<br>**-DETECTOR:**<br>**-COLUMN:** | 180 °C<br>200 °C<br>4 min @ 120 °C; 16 °C/min; 4 min @ 190 °C |
| **CONTROLS:** | collect urine from unexposed workers; pool and freeze the control urine | **COLUMN:** | 3 m x 2-mm ID glass, 2% diethylene glycol adipate/Anakrom Q, 60/80 mesh |
| | | **CARRIER GAS:** | $N_2$, 25 mL/min |
| | | **CALIBRATION:** | analyte in control urine; nitrobenzene internal standard |
| | | **RANGE:** | 2 to 300 µg phenol/mL urine;<br>2 to 500 µg p-cresol/mL urine |
| | | **ESTIMATED LOD:** | 0.5 µg/mL urine |
| | | **RECOVERY:** | (1) 94% @ 15 mg/mL; (2) 95% @ 50 µg/mL |
| | | **PRECISION ($\bar{S}_r$):** | (1) 0.128; (2) 0.091 |
| | | **ACCURACY:** | (1) ± 31.0%; (2) ± 22.8% |

**APPLICABILITY:** Phenol and p-cresol occur normally in urine. This method is useful in screening workers exposed to phenol, p-cresol, and benzene. The chief metabolite of benzene is phenol [1]. Workers exposed 8 h to 25 ppm benzene excreted abou t 150 mg phenol/L urine [2].

**INTERFERENCES:** o-Phenylphenol has a GC retention time similar to that of phenol. A careful work history/questionnaire is suggested.

**OTHER METHODS:** This method replaces P&CAM 330 [3]. A nonspecific colorimetric method yields 50% higher phenol concentrations with normal urine than does this method [4].

**REAGENTS:**

1. Phenol calibration stock solution, 2 mg/mL. Accurately weigh 200 mg phenol* and dissolve in distilled water. Dilute to 100 mL. Stable 14 days at 25 °C.
2. p-Cresol calibration stock solution, 5 mg/mL. Accurately weigh 500 mg p-cresol* and dissolve in methanol.* Dilute to 100 mL. Stable 14 days at 25 °C.
3. Diethyl ether.*
4. HCl, concentrated, or perchloric acid, 70%.*
5. Sodium sulfate, granular, anhydrous.
6. Thymol, USP.
7. Internal standard, 0.6 mg/mL. Dissolve 30 mg nitrobenzene* in 50 mL methanol.
8. Methanol.*
9. Nitrogen, purified.
10. Hydrogen, purified.
11. Air, filtered.
12. Dry ice.

* See SPECIAL PRECAUTIONS.

**EQUIPMENT:**

1. Bottles, polyethylene, screw-top, 125-mL.
2. Gas chromatograph with FID, integrator and column (page 8305-1).
3. Centrifuge tubes, 15-mL, graduated, glass-stopper.
4. Syringe, 10-µL, readable to 0.1 µL.
5. Volumetric flasks, 100-mL.
6. Pipets, Pasteur.
7. Pipets, 1-, 2- and 5-mL.
8. Mixer, vibration.
9. Culture tubes, disposable, 10 x 75-mm.
10. Water bath, 95 °C.
11. Ice bath or freezer.

**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. Diethyl ether and methanol are fire risks. Phenol, p-cresol, methanol and nitrobenzene are toxic and can be absorbed through the skin. Hydrochloric acid and perchloric acid can damage the skin. Wear gloves and eye protection. Work in a fume hood. Handle perchloric acid only in a perchloric acid hood.

**SAMPLING:**

1. Collect 50 to 100 mL urine in a 125-mL polyethylene bottle containing a few crystals of thymol.
   NOTE: Collect two urine samples for each worker, one prior to exposure and one after exposure. Also, collect and pool control urine samples from unexposed workers.
2. Close the bottle immediately after sample collection and swirl gently to mix.
3. Freeze the urine and ship in dry ice in an insulated container.

**SAMPLE PREPARATION:**

4. Thaw urine sample.
5. Determine creatinine (g/L urine) in an aliquot of the urine [5].
6. Pipet 5.0 mL urine into a 15-mL centrifuge tube.
7. Add 1 mL conc. HCl or 5 drops 70% perchloric acid. Mix well.
8. Stopper loosely. Heat in a water bath at 95 °C for 1.5 h.
9. Remove from water bath. Add 10 µL internal standard. Adjust volume in the centrifuge tube to 10 mL with distilled water.
10. Pipet 2 mL diethyl ether into the tube. Stopper and shake vigorously for 1 min. Cool the tube to 0 °C and allow the phases to separate.

11. Transfer ca. 0.5 mL of the clear ether layer to a culture tube. Add a few milligrams of $Na_2SO_4$ and mix. Cap the tube and keep it at 0 °C prior to measurement to avoid evaporation.

**CALIBRATION AND QUALITY CONTROL:**

12. Calibrate daily with combined working standards containing 0.5 to 300 µg phenol/mL solution and 0.5 to 500 µg p-cresol/mL solution.
    a. Add known amounts of phenol and p-cresol calibration stock solutions to pooled control urine in 100-mL volumetric flasks and dilute to the mark with pooled control urine.
    b. Process 5 mL of each working standard using the same procedure as for the samples (steps 6 through 11).
    c. Analyze working standards with urine samples and pooled control urines.
    d. Plot separate calibration graphs for phenol and p-cresol (ratio of peak area of analyte to peak area of nitrobenzene vs. µg analyte/mL solution).

**MEASUREMENT:**

13. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 8305-1. Inject an aliquot of the extract from step 11 using solvent flush technique.
14. Measure peak areas. Divide the peak areas of phenol and p-cresol by the peak area of nitrobenzene on the same chromatogram.

**CALCULATIONS:**

15. Determine the phenol and p-cresol concentrations (µg/mL) in the urine sample from the calibration graphs.
16. Calculate the concentrations of phenol and p-cresol per gram of creatinine in the urine sample by dividing by the creatinine value obtained in step 5. Compare the results obtained on the pre- and post-shift samples for each worker.

**GUIDES TO INTERPRETATION:**

The normal range for phenol found in this laboratory for human controls not exposed to benzene, phenol, or p-cresol was 4.5 to 20.7 mg phenol/g creatinine. The normal range found for p-cresol was 5.5 to 65 mg/g creatinine. It must be emphasized that laboratories should establish their own normal ranges using urine specimens from personnel not exposed to benzene, phenol, p-cresol or excessive amounts of dietary sodium benzoate (used as a preservative in some foods). Lauwerys [6] reported "tentative maximum permissible values" of 45 mg phenol/g creatinine for benzene exposures and 300 mg phenol/g creatinine for phenol exposures. No values were reported for p-cresol. The ACGIH Biological Exposure Index is 250 mg phenol/g creatinine [7].

**EVALUATION OF METHOD:**

Ten spiked urine specimens containing phenol and p-cresol at concentrations of 10 and 50 µg/mL urine, respectively, were analyzed for each analyte. Precision ( $\bar{S}_r$) for the ten spiked replicate urine samples was 0.128 for phenol and 0.091 for p-cresol.

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