

# HIPPURIC ACID in urine

8300

$C_6H_5CONHCH_2COOH$

MW: 179.18

CAS: 495-69-2

RTECS: MR8150000

METHOD: 8300, Issue 2

EVALUATION: PARTIAL

Issue 1: 15 February 1984

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**BIOLOGICAL INDICATOR OF:** exposure to toluene.

**SYNONYMS:** N-benzoylglycine

SAMPLING		MEASUREMENT	
<b>SPECIMEN:</b>	urine, end of shift after 2 days exposure	<b>TECHNIQUE:</b>	VISIBLE ABSORPTION SPECTROPHOTOMETRY
<b>VOLUME:</b>	50 to 100 mL in 125-mL plastic bottle	<b>ANALYTE:</b>	complex of hippuric acid and benzenesulfonyl chloride
<b>PRESERVATIVE:</b>	a few crystals of thymol; keep at about 4 °C	<b>WAVELENGTH:</b>	410 nm
<b>SHIPMENT:</b>	pack in insulated shipper with bagged refrigerant; ship by air express	<b>PATH LENGTH:</b>	1 cm
<b>SAMPLE STABILITY:</b>	stable 1 day @ 20 °C; 1 week @ 4 °C; and 2 months @ -20 °C	<b>CALIBRATION:</b>	aqueous hippuric acid standards
<b>CONTROLS:</b>	collect pre-shift urines as well as urines from non-exposed controls	<b>QUALITY CONTROLS:</b>	frozen pooled urine; correct for creatinine content
		<b>RANGE:</b>	0.005 to 0.5 g/L (1:5 urine dilution)
		<b>ESTIMATED LOD:</b>	0.002 g/L
		<b>PRECISION (<math>\bar{S}_r</math>):</b>	0.06
		<b>ACCURACY:</b>	not determined

**APPLICABILITY:** Toluene is metabolized by the body and is excreted in the urine as hippuric acid, the glycine conjugate of benzoic acid. This method is useful in screening workers exposed to toluene in the absence of xylene or styrene. The latter 2 compounds produce metabolites that are measured as "hippuric acid."

**INTERFERENCES:** In addition to positive interferences from styrene and xylene in the workplace, the ingestion of sodium benzoate in food, salicylic acid, or aspirin by the worker will produce a positive interference. A careful work history/ questionnaire is suggested.

**OTHER METHODS:** This method replaces P&CAM 327 [1] with minor revisions. Method 8301 (Hippuric and Methyl Hippuric Acids in urine) is a specific HPLC method and can be used in the presence of xylene, styrene, salicylic acid and aspirin. Other biological monitoring methods include measurement of blood toluene and alveolar air toluene [2].

**REAGENTS:**

1. Calibration stock solution, 0.5 g/L. Dissolve 50 mg hippuric acid in 100 mL distilled water. Stable one month at 25 °C.
2. Benzenesulfonyl chloride.\*
3. Pyridine, reagent.\*
4. Thymol, USP.
5. Ethanol, absolute.

\* See Special Precautions.

**EQUIPMENT:**

1. Bottles, polyethylene, 125-mL.
2. Spectrophotometer, 1-cm light path, to read at 410 nm, band width  $\leq$  10 nm, with 1-cm cuvettes.
3. Centrifuge, clinical, table top.
4. Centrifuge tubes, 15-mL, conical.
5. Pipets, serological, 0.5-, 1.0-, and 5.0-mL
6. Pipet bulb.
7. Mixer, vibration.
8. Volumetric flasks, 10- and 100-mL.
9. Graduated cylinder, 10-mL.

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**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. Work with pyridine and benzenesulfonyl chloride only in a fume hood.

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**SAMPLING:**

1. Collect a spot urine sample of 50 to 100 mL in a 125-mL polyethylene bottle containing a few crystals of thymol.  
NOTE: Take the sample at the end of the second day of suspected exposure to toluene. Also take pre-exposure samples and samples from non-exposed workers as controls.
2. Pack the samples in an insulated shipper with bagged refrigerant.

**SAMPLE PREPARATION:**

3. Thaw the urine samples, if frozen.
4. Perform a creatinine determination on an aliquot of the urine (e.g., [3]).
5. Dilute 1 volume urine with 4 volumes distilled water.

**CALIBRATION AND QUALITY CONTROL:**

6. Prepare working standards over the range of 0.005 to 0.5 g/L by dilution of calibration stock solution. Working standards are stable for one week at room temperature.
7. Analyze the working standards (steps 10 through 15).
8. Include a frozen pooled urine control with each measurement run.
9. Plot the absorbance at 410 nm against hippuric acid concentration (g/L) in the working standard to prepare the calibration graph.

**MEASUREMENT:**

10. Mix 0.5 mL diluted urine and 0.5 mL pyridine in a conical centrifuge tube.
11. Add 0.2 mL benzenesulfonyl chloride and mix for about 5 sec on a vibration mixer.
12. Let stand for 30 min at 20 to 30 °C.

13. Stop the reaction by adding 5.0 mL ethanol, followed by mixing on a vibration mixer.
  14. Centrifuge at 1500 to 2000 RPM (full speed) for 5 min to reduce turbidity.
  15. Remove supernatant with pipet and place in a 1-cm cuvette. Read absorbance at 410 nm using ethanol to zero the instrument.
- NOTE: If absorbance is above the calibration range, discard the sample and start a new sample (beginning at step 5) with greater dilution.

#### CALCULATIONS:

16. Determine the concentration,  $C_s$  (g/L), of hippuric acid corresponding to the absorbance of the sample from the calibration graph.
17. Calculate the concentration of hippuric acid/g creatinine in the urine sample,  $C$  (g/g creatinine), using the dilution factor,  $D$  (usually 5), from step 5 and the creatinine value,  $C_r$  (g creatinine/L urine), from step 4:

$$C = \frac{C_s \times D}{C_r}, \text{ g hippuric acid/g creatinine.}$$

#### GUIDES TO INTERPRETATION:

1. Tomukuni, et al. [4] reported a normal range in 20 non-exposed adults as  $0.44 \pm 0.20$  g/L (equivalent to approximately 0.7 g/g creatinine).
2. Lauwreys [2] reported a normal range of 1.5 g/g creatinine and a "tentative maximum permissible value" of 2.5 g/g creatinine.
3. Pagnotto, et al. [5] reported that exposure to 100 ppm toluene would produce end-of-shift urinary hippuric acid levels of 4 g/L (equivalent to about 5 g/g creatinine).
4. Urine from non-exposed workers should be collected, as well as pre-shift specimens from exposed workers because levels of hippuric acid in urine vary widely.

#### EVALUATION OF METHOD:

Precision ( $s_r$ ) for within-run and day-to-day runs over the analytical range was found to average 0.06. No method comparison studies have been conducted.

#### REFERENCES:

- [1] NIOSH Manual of Analytical Methods, 2nd Ed., Vol. 6, Method P&CAM 327, U.S. Department of Health and Human Services, Publ. (NIOSH) 80-125 (1980).
- [2] Lauwerys, R.R. Industrial Chemical Exposure: Guidelines for Biological Monitoring, Biomedical Publications, Davis, California, 57-65 (1983).
- [3] Tietz, N.W. Fundamentals of Clinical Chemistry, 2nd ed., pp. 994-999, W.B. Saunders Co., Philadelphia, PA (1976).
- [4] Tomokuni, K. and M. Ogata. Direct Colorimetric Determination of Hippuric Acid in Urine, Clin. Chem. **18**, 349-351 (1972).
- [5] Pagnotto, L.D. and L.M. Lieberman. Am. Ind. Hyg. Assoc. J., **28**, 129-134 (1967).

#### METHOD REVISED BY:

Frederick C. Phipps, NIOSH/DBBS.