o-CRESOL in URINE

8321

$CH_3C_6H_4OH$ MW: 108.14 CAS: 95-48-7 RTECS: GO6300000

METHOD: 8321, Issue 1 **EVALUATION:** FULL **Issue 1:** 18 March 2016

OSHA: N/A
NIOSH: N/A
Because data on exposure limits and guidelines may change over time, NIOSH recommends referring to references 1-4 for updated limits and guidelines concerning o-cresol and its use as a marker for other compounds.

PROPERTIES: colorless solid; MP 31 °C; BP 191 °C
Density (35 °C) = 1.0327 g/mL [5]

SYNONYMS: *ortho*-cresol, 2-cresol, *o*-cresylic acid, 1-hydroxy-2-methylbenzene, 2-hydroxytoluene, 2-methylphenol

SAMPLING

SPECIMEN: Urine

VOLUME: 50-100 mL in screw cap bottle; minimum of 10 mL

SHIPMENT: < 6 ºC

SAMPLE STABILITY: Stable at least 30 days at 4 °C [6]

CONTROLS: Collect urine from unexposed workers, pool and refrigerate

ACCURACY

ESTIMATED LOD: 0.01 µg/mL [6]

RANGE STUDIED: 0.0214 to 30.4 µg/mL [6]

ACCURACY: ± 21% [6]

BIAS: -0.0952 [6]

OVERALL PRECISION ($\hat{S}_{rT}$): 0.0771 [6]

RECOVERY: 92 –112 % [6]

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY/MASS SPECTROMETRY with SELECTED ION MONITORING (GC/MS-SIM)

ANALYTE: *o*-Cresol

TREATMENT: Acid hydrolysis and liquid/liquid extraction

INJECTION VOLUME: 2 µL

TEMPERATURE
-INJECTION: 265 °C
-DETECTOR: 285 °C
-COLUMN: 50 °C(hold 2 min); 50 to 150 °C @ 10 °C/min; 150 to 310 °C @ 20 °C/min (hold 5 min)

CARRIER GAS: Helium, at 1.3 mL/min

COLUMN: Capillary, fused silica, phenyl arylene polymer virtually equivalent to (5% phenyl)-methylpolysiloxane (30 m x 0.32 mm ID, 0.5 µm film); close equivalent to USP G27

MS PARAMETERS: SIM (ions m/z 108, 107, 77 and 123) Quantifying ions: o-cresol: 108, nitrobenzene: 123. Confirmation ions: o-cresol: 107, nitrobenzene: 77

DWELL TIME: 30 msec

CALIBRATION: *o*-Cresol spiked in control urine and nitrobenzene as internal standard

APPLICABILITY: This method can be used for the determination of total o-cresol in urine specimens. Cresols are excreted in urine primarily as conjugates. This method uses an acid hydrolysis step to convert the conjugates to free o-cresol. (See Evaluation of Method Section.) Exposure to o-cresol will cause elevated urinary levels; however, o-cresol is a metabolite produced from toluene exposure. Not only would toluene exposure interfere with determining o-cresol exposure, but measuring o-cresol in urine is one of the recommended methods for determining toluene exposure. Recommended levels for using o-cresol to measure toluene exposure (at the time of publication of this method) include: ACGIH-TLV, 0.3 mg/g creatinine [1]; DFG-BAT, 1.5 mg/L [2]; and SUVA-VBT, 0.5 mg/L [3].

INTERFERENCES: None noted in the analytical method, but background levels of 0.032 – 0.070 µg/mL o-cresol have been observed [7-9]. These values are near and slightly above the limit of quantitation. Exposure to toluene will cause higher levels of urinary o-cresol as discussed above. p-Cresol is a normal component of human urine [10] and may, along with m-cresol, also be a co-exposure [11-12]. These two compounds have similar mass spectra to o-cresol but are chromatographically resolved under the conditions of this method [6].

OTHER METHODS: There are numerous literature methods for the determination of o-cresol in urine [10], but standardized methods from governmental agencies or consensus standards organizations are not currently available. NIOSH 8305 is a similar but only partially-validated method for phenol and p-cresol [11]. An older validated method using steam distillation and HPLC-UV detection for ~30 related compounds that includes o-cresol can be found in the German MAK collection [12].

REAGENTS:

1. *o*-Cresol* (≥99% purity) primary stock solution (PSS) about 10,000 µg/mL (0.100 g of *o*-cresol in 10 mL of methanol). Store at -10 °C ± 2 °C
2. *o*-Cresol intermediate solutions of 1,000 and 100 µg/mL (0.5 and 0.05 mL of PSS diluted to 5.0 mL with methanol). Store at -10 °C ± 2 °C
3. Human urine* provided by non-exposed individuals, store at < 6 °C**
4. Methanol*, GC grade or better
5. Hydrochloric acid*, concentrated, 33-38%, ACS reagent grade or better
6. Nitrobenzene (≥99% purity) internal standard (IS) solution of 500 µg/mL (0.125 g of nitrobenzene in 250 mL of methanol). Store at 10 °C ± 2 °C
7. Water, ASTM Type II [13]
8. Methyl tert-butyl ether* (MTBE), HPLC grade or better
9. Sodium sulfate, granular, anhydrous, ≥99% purity.
10. Helium, UHP or Grade 5

*See SPECIAL PRECAUTIONS.
**Human urine recommended due to evidence of column problems caused by synthetic urine.

EQUIPMENT:

1. GC-MS capable of selected ion monitoring with data system, autosampler, and column (page 8321-1)
2. Flasks, glass, volumetric: 5, 10 and 250 mL
3. Syringes, glass: 10, 50, 100, 500 and 2,500 µL
4. Tubes, centrifuge, 15-mL graduated polypropylene, with caps
5. Pipetter 1-10 mL, with disposable tips
6. Pasteur pipettes, glass
7. Water bath, 95 °C
8. Wash bottle
9. Vials, autosampler, amber glass
10. Cold storage for -10 °C and 4 °C
11. Bottles, 125 mL polyethylene
12. Analytical balance, to ±0.0001 g.

SPECIAL PRECAUTIONS: Wear gloves, lab coat, and safety glasses while handling all chemicals and human urine products. Disposable plastic, glass, and paper (pipet tips, gloves, etc.) that contact urine should be placed in a biohazard container. Standard precautions should always be used when handling bodily fluids and/or extracts of bodily fluids [14]. Handle urine samples and urine extracts using proper gloves. All work should be performed in a fume hood. Methanol and MTBE are both flammable. Hydrochloric acid is an extremely corrosive chemical capable of severe tissue damage.

SAMPLING:

1. Collect 50-100 mL urine in a 125 mL polyethylene bottle. Collect 2 urine specimens for each worker: one specimen before exposure and one specimen after.
2. Collect and pool urine specimens from unexposed workers to be used for controls.
3. Tightly cap each bottle and ship refrigerated or frozen in an insulated container to maintain the temperature at 6 °C or below.

SAMPLE PREPARATION:

4. Allow urine to reach room temperature and mix thoroughly.
   NOTE: If desired, remove an aliquot of urine to determine creatinine levels.
5. Dispense a 5-mL aliquot of the urine specimen into a 15-mL graduated centrifuge tube.
6. Using a Pasteur pipette, add 1 mL of concentrated HCl, up to the 6 mL calibration mark in the tube.
7. Cap the tube. Shake vigorously for one minute.
8. Place the tube in a water bath at 95 °C for 1.5 hr.
   NOTE: Covering the water bath may be required to maintain the temperature at 95 °C.
9. Remove the tube from the bath and let it cool to room temperature.
10. Add 250 µL of the nitrobenzene IS solution.
11. Add water (ASTM Type II) to the tube, filling it to the 10 mL calibration mark.
12. Pipet 2 mL of MTBE to the tube and cap it.
13. Shake the tube vigorously for 2 min.
14. Allow the phases to separate.
15. Transfer the top organic phase to an amber GC vial containing approximately 0.2 g of anhydrous sodium sulfate (to ensure the dryness of the organic fraction).
16. Cap the vials and store at <6 °C until GC-MS analysis.

CALIBRATION AND QUALITY CONTROL:

17. Calibrate daily with nine working standards containing *o*-cresol at approximately the following concentrations: 0.04, 0.1, 0.2, 1, 2, 10, 20, 40 and 60 µg/mL.
    a. Prepare the standards by adding measured amounts of *o*-cresol intermediate stock solutions to centrifuge tubes containing 5 mL of control urine.
    b. Process the standards following the same procedure used for the samples (steps 4-16).
    c. Prepare at least one method blank for every 20 samples by taking an aliquot of 5 mL control urine and processing it in the same manner as the samples (steps 4-16).
    d. Prepare at least two levels of quality control (QC) samples using a separate source of *o*-cresol and prepare in the same manner as the calibration standards within the analytical range. These levels should be at roughly 10 times the limit of quantitation (LOQ) and 100 X LOQ. Analyze at least two QC samples of different concentration for every 20 samples.
18. Prepare a calibration graph by plotting, for each working standard, the normalized analyte response (peak area of analyte divided by the peak area of the internal standard on the same chromatogram) on the y-axis vs. µg of analyte/mL of urine on the x-axis. The simplest model that adequately describes the data should be used, but either a linear (most likely 1/X weighted because of the range of the calibration curve) or a quadratic model may be utilized in processing the analytical data. Because there may be detectable levels of *o*-cresol in the pooled urine blanks, before plotting the calibration graph, subtract the normalized analyte response of the pooled urine blank from the normalized analyte response of each working standard. The standard curve should have a coefficient of determination (r^2) of equal to or greater than 0.98 to be acceptable for use. Furthermore, when each standard is substituted back into the calibration equation, the value should be within ±20% of the expected value.
19. QC values should be within ±20% of the spiked values. If not, the batch is considered out of control, the batch data discarded, and corrective actions taken before more samples are analyzed.

MEASUREMENT AND CALCULATIONS:

20. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 8321-1.
21. Set the mass spectrometer according to manufacturer's recommendations, to conditions given on page 8321-1, and to SIM for ions *m/z* 77, 107, 108, and 123.
22. Inject 2 µL of each sample, blank and QC sample.
23. Measure peak areas for *o*-cresol and nitrobenzene in the chromatograms. Divide the peak area of *o*-cresol by the peak area of nitrobenzene in the same chromatogram.

CALCULATIONS:

24. Determine concentration of *o*-cresol from the calibration curve produced in step 18.

EVALUATION OF METHOD:

This method was evaluated at five concentration levels over the range 0.0214 – 2.14 µg/mL. This range represents from 1 x LOQ to 100 x LOQ. Six replicates were analyzed at each level. The average recoveries at the various levels ranged from 86.8 to 118.4%. The limit of detection (LOD) and LOQ study was performed by analyzing a series of standards ranging from 0.0107 to 64.1 µg/mL, fitting the data to a quadratic curve, and estimating the values using "Burkart's Method" [15]. The value obtained for the LOD by this method (0.009 µg/mL) was lower than the lowest standard in the determination (0.0107 µg/mL), so the LOD used for the method was the value of the lowest standard, 0.01 µg/mL. A long-term storage study was carried out at the 30 x LOQ level: pooled urine specimens spiked with *o*-cresol at 0.854 µg/mL were stored at 4 °C for 1, 7, 10, 14, 21 and 30 days and then analyzed. The recoveries across the entire study (1-30 days) were between 92-112%. Another set of specimens stored at room temperature were analyzed after 1 and 7 days, producing recoveries ranging from 90-102% [6]. The analytical range of the method was extended during a secondary laboratory validation step. Two concentration levels were evaluated (5.06 and 30.30 µg/mL) by analyzing five replicates at each level. These levels showed recoveries of 100.2 and 92.8% with relative standard deviations of 0.63 and 1.40% respectively [6].

In order to minimize confusion among users of NIOSH Method 8321, the authors would like to re-emphasize that *p*-cresol is an endogenous human metabolite produced from protein breakdown and will always be found in human urine [10]. The mass spectrum of *p*-cresol is quite similar to that of *o*-cresol and so there is some possibility of peak misidentification. Fortunately, while the peaks are near each other in the chromatogram under the method conditions, the *p*-cresol peak elutes later, is adequately resolved, and thus will not interfere with *o*-cresol analyses [6].

Discussion concerning acid hydrolysis step: The efficiency of the acid hydrolysis step in this procedure was not evaluated. A common method of converting these conjugates (glucuronides and sulfates) back to the parent compounds is heating the sample with a mineral acid. Several literature examples that employ hydrochloric acid under very similar conditions can be found [8,9,12,16-18]. A common thread among these methods is that none of them appear to investigate the efficiency of the acid hydrolysis step. Fustinoni et al. did investigate the hydrolysis step both in terms of amount of acid used and reaction time required [19]. They found that 50 µL of concentrated HCl per 300 µL of urine and reacting for 60 min at 100 °C gave yields of greater than 97%. NIOSH Method 8321 uses 1 mL concentrated HCl per 5 mL urine, heating at 95 °C for 1.5 h, which are nearly identical conditions. These similar conditions should assure adequate efficiency of the acid hydrolysis step and have proven to not be detrimental to the *o*-cresol [6].

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