2,5-HEXANEDIONE in URINE

8318

$CH_3COCH_2CH_2COCH_3$ MW: 114.14 CAS: 110-13-4 RTECS: MO3150000

METHOD: 8318, Issue 1 **EVALUATION:** FULL **Issue 1:** 15 August 2017

NIOSH and OSHA: NA
Because data on exposure limits and guidelines may change over time, NIOSH recommends referring to the following sources for updated limits and guidelines concerning 2,5-hexanedione as well as its use as a marker for other compounds [1-3].

PROPERTIES: Clear liquid; MP -5.5 °C; BP 191 °C; d^{25} 0.973 g/mL; VP 0.43 mm Hg at 20 °C [4]

BIOLOGICAL INDICATOR OF: exposure to n-hexane and methyl n-butyl ketone

SYNONYMS: acetonyl acetone; diacetonyl; alpha, beta-diacetylethane; 1,2-diacetylethane; 2,5-diketohexane; 2,5-dioxohexane

SAMPLING

SPECIMEN: two urine samples (one at the beginning of work week and one end of shift late in the work week)

VOLUME: complete void stored in a polyethylene screw-cap bottle

PRESERVATIVE: none

SHIPMENT: refrigerated or frozen in well-insulated container

SAMPLE STABILITY: stable 7 days @ 24 °C and at least 30 days @ 4 °C [5]

CONTROLS: collect and pool urine from matched population of unexposed workers if possible then refrigerate immediately

ACCURACY

RANGE STUDIED: 2.1 - 212 mg/L [5]

BIAS: -0.0189

OVERALL PRECISION ($\hat{S}_{rT}$): 0.0235 [5]

ACCURACY: ± 5.6%

RECOVERY: 102% (Day 32) [5]

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY, FLAME IONIZATION DETECTOR

ANALYTE: 2,5-hexanedione (2,5-HD)

TREATMENT: acid hydrolysis; dichloromethane extraction

INJECTION VOLUME: 3µL; splitless for 0.7 min

TEMPERATURE
- **-INJECTION:** 200 °C
- **-DETECTOR:** 250 °C
- **-COLUMN:** 45 °C (2 min hold); 45 to 60 °C @ 5 °C/min; 60 to 140 °C @ 10 °C/min; 140 to 220 °C @ 50 °C/min; 220 °C (5 min. hold); final hold time may be extended if necessary.

CARRIER GAS: Helium, at 15 psi head pressure

COLUMN: Capillary, fused silica, polyethylene glycol, 30 m x 0.32 mm ID, 0.5 µm film

CALIBRATION: analyte in control urine; 2-methyl-3-heptanone or other appropriate internal standard

ESTIMATED LOD: 0.2 mg/L in pooled urine [5]

APPLICABILITY: Can be used in monitoring the exposure of workers to n-hexane or methyl n-butyl ketone. This method measures "total" amount of 2,5-HD, not the "free" amount. See further discussion in Other Methods section.

INTERFERENCES: Other compounds besides n-hexane (e.g. methyl n-butyl ketone) can be metabolized into 2,5-HD. Acid hydrolysis can convert other metabolites (e.g. 4,5-dihydroxy-2-hexanone) into 2,5-HD, giving values higher than non-hydrolysis methods [6-8]. Coexposure to toluene and methyl ethyl ketone has been shown to inhibit n-hexane metabolism [9]. General population background levels of 2,5-HD vary by age and gender but average 0.4 mg/L [10].

OTHER METHODS: This method is based on the acid hydrolysis method of Iwata et al. [9], and Fedtke and Bolt [8] as described by Kawai et al. [11]. As discussed in greater detail in the Backup Data Report [5], this method uses an acid hydrolysis step to convert some of the intermediate metabolites to 2,5-HD, a measure of "total" 2,5-HD. A non-hydrolysis method, which measures the "free" 2,5-HD, was not investigated in this work. The "free" amount is associated with the Biological Exposure Index [1]. Other exposure values are more associated with the "total" amount found [2,3] and still others give the option of either approach [12]. Users should recognize the advantages and limitations of each approach when selecting a methodology. More on this can be found in the Evaluation of Method section and the backup data report [5].

REAGENTS:

1. Control urine, collected and pooled from unexposed workers*
2. 2,5-hexanedione stock solutions: Prepare by diluting the appropriate amounts of the pure analyte in methanol. Prepare working standards by diluting aliquots of the stock solution with pooled urine*
3. Extraction Solution: Dissolve 5 mg of the internal standard (2-methyl-3-heptanone) into dichloromethane; bring to 1.0 L total volume*
4. Hydrochloric acid (HCl), concentrated* (Trace metal grade or higher)
5. Methanol* (HPLC grade or higher)
6. Dichloromethane* (ACS reagent grade or higher)
7. Helium, purified
8. Hydrogen, prepurified
9. Air, filtered

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Bottles, polyethylene screw-top, 125-mL
2. Gas chromatograph, with flame ionization detector, data system, and column (page 8318-1)
3. Vials, autosampler, glass, 2-mL, PTFE-lined crimp caps
4. Bagged refrigerant or dry ice
5. Pipettes, 5-, 2-, and 1-mL, plastic or serological
6. Heated water bath
7. Centrifuge
8. Culture tubes, glass, 13 x 100 mm with PTFE-lined screw caps
9. Syringes, 10-, 100-mL
10. Pipets, glass
11. pH meter
12. Magnetic stirrer
13. Volumetric flasks, glass, 1-L, 10-mL

SPECIAL PRECAUTIONS: Standard precautions should always be used when handling bodily fluids and/or extracts of bodily fluids [13]. Handle urine specimens and urine extracts using powder-free latex or nitrile gloves. All work should be performed in a fume hood since all chemicals are respiratory irritants and some may cause narcotic effects. Inhalation of large amounts of methanol or dichloromethane can cause unconsciousness and even death. 2,5-HD is a neurotoxin at high exposure levels. Methanol is a fire hazard and causes blindness or death if ingested. Hydrochloric acid is an extremely corrosive chemical capable of severe tissue damage.

SAMPLING:

1. Collect a complete void of urine in a 125-mL polyethylene bottle. Collect two urine specimens from each worker: one specimen at the beginning of the work week and one specimen at end of shift late in the work week.
2. Collect and pool urine from unexposed workers to be used for controls.
3. Tightly cap each bottle and ship refrigerated or frozen in a well-insulated container.
   NOTE: Commercial shippers have special labeling requirements for packages containing biological samples and dry ice.

SAMPLE PREPARATION:

4. Allow urine specimens to reach room temperature.
   NOTE: If desired, remove an aliquot of urine to determine creatinine levels (g/L urine).

5. Pipet 5.0 mL of urine into a 13 x 100 mm culture tube or other appropriate container.
6. Utilizing a magnetic stirrer, add concentrated HCl until the pH of the urine is within the desired range (0.5 to 1.0).
   NOTE: An exact control of the sample's pH is critical for consistent and reproducible results [11].
7. Cap tube and heat in a water bath at 100 °C for 30 min.
8. Remove from water bath and allow to cool completely before proceeding.
9. Pipet 2 mL of the extraction solution, containing the internal standard, into the tube.
10. Cap and shake vigorously for 1 min. Allow the phases to separate. A centrifuge is recommended to aid in separation.
11. Remove a portion of the extraction solution (bottom layer) using a glass pipet and transfer it to a 2-mL glass GC autosampler vial. Cap and analyze.

CALIBRATION AND QUALITY CONTROL:

12. Calibrate daily with at least six working standards covering the concentration range of the specimens.
13. Prepare each working standard by diluting a known amount of 2,5-HD stock solution into enough pooled urine to make a total of 5.0 mL.
14. Prepare at least one pooled urine blank by transferring 5.0 mL of pooled urine (the same pooled urine used for creating the working standards) into a culture tube.
15. Prepare at least two levels of quality control (QC) spikes of 2,5-HD in pooled urine to be analyzed with each analysis batch. These levels should be at approximately 10 times the limit of quantitation (LOQ) and 200 times the LOQ, but can be adjusted to better suit the anticipated levels of the specimens. QC samples should be analyzed with every batch such that they constitute 10% of the specimen batch.
16. Process the 5 mL of each working standard and each pooled urine blank using the same procedure as for the specimens (steps 5 through 11).
17. Analyze the working standards, the pooled urine blanks, the QC samples, and the samples together.
18. QC values should normally 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 specimens are analyzed.

MEASUREMENT:

19. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 8318-1. Typical retention times for the two compounds under these conditions are 4.7 min for the internal standard and 10.5 min for 2,5-HD.
20. Inject each of the standards, QC samples, samples, and blanks.
21. Measure peak area. Normalize analyte response by dividing the peak area of the analyte by the peak area of the internal standard on the same chromatogram.
22. 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. mg of analyte/L of urine on the x-axis. The simplest model that adequately describes the data should be used but either a linear (mostly likely 1/x weighted because of the range of the calibration curve) or a quadratic model may be utilized in processing the analytical data.
    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 plugged back into the calibration

equation, the value should be within ±20% of the expected. Acid hydrolysis can create detectable amounts of 2,5-HD in 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.

CALCULATIONS:

23. Determine the 2,5-HD concentration, C_s (mg/L) in the urine specimen from the calibration graph created in step 16.

24. If desired, calculate the concentration of 2,5-HD in milligrams per gram of creatinine C (mg/g) in the urine specimen by dividing by the creatinine value C_r (g creatinine/L urine) obtained from the aliquot removed in step 4. Compare the results from before and after exposure. Also compare the results of the "exposed" group to that of the control group.

$$C = \frac{C_s}{C_r}$$

EVALUATION OF METHOD

This method was evaluated over the ranges specified on page 8318-1. These ranges represent from 3 times the LOQ to 300 times the LOQ. Seven replicates were analyzed at each level. The average recoveries at the various levels ranged from 97% to 103%. The limit of detection (LOD) and Limit of Quantification (LOQ) were determined by preparing in duplicate and analyzing a series of standards with the data fitted to a quadratic curve. The LOD and LOQ were estimated according to Burkart's Method [14]. A long-term storage study was carried out at the 10 x LOQ level. Pooled urine samples spiked with the analyte were stored at 4 °C for 1, 4, 7, 10, 21, or 32 days and then analyzed. All recoveries were nearly 100%. Spiked pooled urine samples were stable after 7 days at room temperature.

The issue of measuring "total" (the 2,5-HD determined after a hydrolysis step) versus "free" (the 2,5-HD determined without a hydrolysis step) is a contentious one among world bodies that develop biomonitoring guidelines. As previously stated, this method uses a hydrolysis step and was not evaluated without it. Therefore, it measures the "total" amount. NIOSH does not desire to step into the debate over which may be better, but just to offer this method as one way to assess exposure. Some brief summaries of the two approaches: "total" produces a larger number, requiring less sensitivity from the analytical methodology but is subject to higher variability resulting primarily from two sources: variability in hydrolysis procedure [11] and production of 2,5-HD from other metabolites in human urine that vary in concentration. The "free" approach gives a smaller number but tends to be more specific for n-hexane and methyl n-butyl ketone exposure as the other metabolites in urine do not interfere, so more sensitivity is required but background levels are greatly reduced.

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METHOD WRITTEN BY:

Corey C. Downs and James B. Perkins, DataChem Laboratories, Inc., Salt Lake City, Utah under NIOSH Contracts CDC-200-95-2955 and CDC-200-2001-08000. Final editing by Dale A. Shoemaker, Ph.D., NIOSH/DART/CEMB.