TOLUENE in Blood

8007

C_7H_8 MW: 92.14 CAS: 108-88-3 RTECS:XS5250000

METHOD: 8007, Issue 1 **EVALUATION: FULL** **Issue 1: 5 March 2013**

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 [1,2].

PROPERTIES: Clear liquid; bp 110.6 °C; VP 28.4 mm Hg at 25 °C; d^{20} 0.8669 g/mL.

BIOLOGICAL INDICATOR OF: Exposure to toluene

SYNONYMS: Methylbenzene, Phenylmethane, Toluol, Methylbenzol

SAMPLING

SPECIMEN: Blood (collected within 12 hours of last exposure)

VOLUME: Fill a 10-mL vacuum specimen tube as completely as possible (to reduce the amount of airspace)

PRESERVATIVE: Sodium citrate (light blue top)

SHIPMENT: Insulated container with bagged refrigerant

SAMPLE STABILITY: Stable at least 30 days at 4 °C [3]

CONTROLS: Collect and refrigerate immediately

ACCURACY

RANGE STUDIED: 0.0208-5.76 µg/mL [3]

ACCURACY: <5% [3]

BIAS: 0.0006

OVERALL PRECISION ($\hat{S}_{rT}$): 0.0157 [3]

SAMPLE STABILITY: 99.0% (Day 30) [3]

MEASUREMENT

TECHNIQUE: HEADSPACE/GAS CHROMATOGRAPHY, FID

ANALYTE: Toluene

HEADSPACE CONDITIONS: Transfer Temp,129 °C; Withdrawal, 0.80 min; Thermostat Time, 30 min; Needle, 120 °C; GC Cycle Time, 34 min; Sample Temp: 99 °C; Pressurize, 4.0 min; Inject, 0.40 min

INJECTION CONDITIONS: Direct injection onto column for 0.4 min, then split flow for the remainder of the run at 130 mL/min

TEMPERATURE-INJECTION: 129 °C
-DETECTOR: 250 °C
-COLUMN: 50 °C (hold for 2 min); 50 to 220 °C @ 10 °C/min; 220 °C (hold for 5 min)

CARRIER GAS: Helium, at 21 psi head pressure

COLUMN: Capillary, fused silica, 75-m x 0.53 mm, coated internally with 3.0 µm film 6% cyano- propylphenyl, 94% dimethylpoly-siloxane, bonded and cross-linked

CALIBRATION: Analyte in control blood; with isobutanol internal standard (IS)

Estimated LOD: 0.006 µg/mL in whole blood [3]

APPLICABILITY: Can be used in monitoring the exposure of workers and drug abusers for toluene.

INTERFERENCES: None identified. The chromatographic separation conditions may be adjusted to correct separation problems.

OTHER METHODS: NIOSH Method 8002 is a partially-evaluated, packed-column GC/FID method with lower accuracy and a higher detection limit. [4] The National Center for Environmental Health (NCEH)/CDC has a method for 31 volatile organic compounds in blood that includes toluene. This NCEH method is somewhat more complicated, comparable in accuracy, and ~1,000 times more sensitive. [5]

REAGENTS:

1. Whole blood*, citrate treated
2. Toluene* (reagent grade, >99.5%)
3. Isobutanol internal standard (IS) as a 20 mg/L solution in water*
4. Water, ASTM Type II
5. Helium, UHP or higher
6. Hydrogen, UHP or higher
7. Air, UHP or higher
8. Toluene calibration standards*
9. Ethylene glycol*

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Gas chromatograph, with FID, column, and data collector (page 8007-1)
2. Headspace sampler, holds up to 40 vials and can thermostat up to 12 vials simultaneously for automated headspace analysis (Any instrument that can achieve the headspace settings specified on p. 8007-1 is acceptable)
3. Bagged refrigerant, and refrigerator
4. Adjustable pipette, 10-mL, tips 10-mL, plastic or serological
5. Headspace vials, 20-mL, with caps and septa
6. Micro-liter syringes, 10-µL , 25-µL, 100-µL, 1-mL
7. Volumetric flasks, 10-mL, 25-mL, 1-L
8. Syringes, twist-on fitting (luer lock style or equivalent), 5-mL, and 20 gauge needles
9. Tubes, vacuum blood collection, 10-mL, citrate coated (light blue top)

SPECIAL PRECAUTIONS: Wear gloves, lab coat, and safety glasses while handling all chemicals and blood products. Disposable plastic, glass, and paper (pipet tips, gloves, etc.) that contact blood should be placed in a biohazard container. Contact with biological samples can have serious health consequences through exposure to hepatitis, HIV, and other diseases. All personnel collecting, handling, or analyzing samples should follow universal precautions [6] and comply with the OSHA bloodborne pathogens standard [7] which includes immunization for hepatitis B. Toluene is extremely flammable. All work should be performed in a fume hood.

SAMPLING:

1. Collect the blood by filling a 10-mL vacuum blood collection tube (light blue top, citrate) as completely as possible in order to keep the air space to a minimum. Be certain to invert the blood tube several (5-10) times to mix the anticoagulant.
   NOTE: If desired, duplicate blood tubes could be drawn to allow for estimation of measurement precision, especially if collecting from numerous participants.
2. Immediately transfer the sample to a refrigerator or cooler (≤ 4 °C.)
3. Ship the sample vial in a well-insulated container equipped with blue ice or other cooling material.

SAMPLE PREPARATION:

4. Allow the blood sample to reach room temperature, then mix gently so as to not produce a froth by inverting the tubes 4 to 5 times.
5. Add 1.5 mL of the IS solution to a 20-mL headspace vial.
6. Extract 1.5 mL of sample from the vacuum vial using a 5-mL syringe with a twist-on fitting (luer lock style or equivalent) or micropipette and transfer the blood to a headspace vial.
7. Cap both sample and headspace vials immediately.
8. Mix the contents of each headspace vial thoroughly.

STOCK STANDARD PREPARATION:

9. Prepare a primary stock standard solution by diluting 20 µL of toluene to 50 mL with ethylene glycol (20 µL/50 mL = 345.7 µg/mL.) Store in a tightly capped glass container having little or no head space. NOTE: Aliquots of toluene should be introduced below the ethylene glycol to prevent loss of toluene.
10. Prepare stock standards by diluting aliquots of the primary standards with ethylene glycol. Suggested levels: 34.6 µg/mL, 3.46 µg/mL, 0.346 µg/mL.

CALIBRATION AND QUALITY CONTROL:

11. Calibrate with at least five working standards in duplicate covering the concentration range of the samples.
    a. Prepare each working standard by adding 1.5 mL of IS solution and 1.5 mL of blank blood to a headspace vial and cap the vial. Spike the mixture in the vial (through the septum using a micro-syringe) with a stock standard solution to the desired concentration of toluene and mix by shaking.
    b. Prepare at least two blanks by repeating step a. but omitting the toluene spike.
    c. Measure the peak areas of toluene and isobutanol in the chromatograms. Subtract the average toluene peak area of the blank from the toluene peak areas of the standards (see NOTE 1.) Divide the peak area of the blank-corrected toluene by the peak area from the isobutanol peak. Prepare a calibration curve of the peak area Std/area IS versus the toluene concentration of the standards.
    NOTE 1: A trace amount of toluene may be present in the blood of some donors. These levels will vary depending upon environmental exposures. If the blanks show the presence of toluene the standards need to be blank corrected, or else the sample results will be biased low. Geometric means for the U.S. population as determined from the National Health and Nutrition Examination Survey may be useful for comparison. [8]
    NOTE 2: It is also highly recommended that a reagent blank or blanks be included in the analysis. Atmospheric toluene in the lab may contribute errors to the measured values. A reagent blank using water, a blank headspace vial, or both of these options will show if the laboratory conditions are free from a quantifiable amount of toluene.
12. Prepare two levels of quality control (QC) samples by spiking toluene into whole blood. These levels could be at ~10 X LOQ and 200 X LOQ, but could be adjusted to better suit the anticipated levels of the sample set. Unspiked samples of the blood used to prepare the QC samples should be analyzed to determine the blank level and the true target level. QC samples should be analyzed with every batch such that they constitute 10% of the sample batch.
13. 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 should be taken before more samples are analyzed. Alternatively, if the method has a long history in the lab allowing enough data for control charts to be constructed, the control charts could serve as the in or out of control decision guide, which could be looser or stricter than the recommended 20%.

MEASUREMENT:

14. Set the gas chromatograph according to manufacturer's recommendations and to conditions given on page 8007-1.
15. Set the headspace sampler according to manufacturer's recommendations and to conditions given on page 8007-1.
16. Inject and analyze samples, standards, QC samples, and blanks.
17. Measure the peak area of both toluene and isobutanol in the chromatograms (do not subtract the blank from the samples). Divide the peak area of the toluene peak by the area from the isobutanol peak.

CALCULATIONS:

18. Determine the concentration (µg/mL) of the toluene in each sample of blood using the calibration curve obtained in step 11c.

EVALUATION OF METHOD:

This method was evaluated over the range specified on page 8007-1. These ranges, 0.0208 to 5.76 µg/mL, represent from 1 x LOQ to 300 x LOQ. Six replicates were analyzed at each level. The average recoveries at the various levels ranged from 97% to 106% for toluene. The LOD and LOQ were determined by preparing a series of duplicate standards. Each series was made up and analyzed on a different day. The resulting data was then fitted to a quadratic curve. The LOD and LOQ were estimated according to Burkart's Method. [9] A long-term storage study was carried out at the 10 x LOQ level. Citrated whole blood samples that were spiked with toluene were stored at 24 °C and 4 °C for 1, 4, and 7 days and for 7,10, 21, and 30 days (respectively,) and then analyzed. Average recoveries were 96% at room temperature and 99% at 4 °C. Room temperature storage is not advised because if the sample clots before it is analyzed, the results will be compromised.

LIMITATION OF METHOD:

Concerning the use of isobutanol as an internal standard: While the boiling points of toluene and isobutanol are similar, their Henry's Law constants and thus their partitioning coefficients are quite different. This could lead to biases if there are differences in polarity in the samples or between the samples and the standards. This is one reason the calibration standards in this method are prepared using blood and not just solvent. An alternative approach could entail the use of an internal standard that partitions similarly to toluene, such as fully-deuterated toluene (toluene-d8). This would cause an increase in the cost of the method per sample (and would need to be validated by the end user), but is mentioned here as an option.

REFERENCES:

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