METHYL ETHYL KETONE, ETHANOL, and TOLUENE in blood 8002

(1) $CH_3CH_2COCH_3$	MW:	72.11	CAS:	78-93-3	RTECS:	EL6475000
(2) CH_3CH_2OH		46.07		64-17-5		KQ6300000
(3) C_7H_8		92.14		108-88-3		XS5250000

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BIOLOGICAL INDICATOR OF: exposure to 2-butanone, ethanol, and toluene.

SYNONYMS: (1) MEK: 2-butanone; methyl ethyl ketone
(2) ethanol: ethyl alcohol
(3) toluene: methyl benzene

BIOLOGICAL SAMPLING

SPECIMEN: venous blood, after 2 or more h exposure

CONTAINER: 5-mL heparin-coated vacuum tube

SHIPMENT: air express @ 4 °C

STABILITY: stable @ 4 °C for 3 weeks

CONTROLS: pre-shift whole blood samples as well as whole blood samples from non-exposed controls

ACCURACY: (1) ±28.6%
(2) ±13.0%
(3) ±26.2%

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY, FID

ANALYTE: MEK, ethanol, toluene (simultaneous)

TEMPERATURE-INJECTION: 150 °C
- DETECTOR: 200 °C
-COLUMN: 85 °C (4 min), 85 to 200°C @ 16 °C/min, 220°C (4 min)

CARRIER GAS: helium, 25 mL/min

COLUMN: glass, 3 m × 2-mm ID, 5% Carbowax 20 M on 100/120 mesh Chromosorb WHP

CALIBRATION: blood standards containing analytes

RANGE: (1) 0.1 to 8 µg/mL
(2) 0.01 to 0.6 mg/mL
(3) 1 to 600 µg/mL

RECOVERIES: (1) 0.90 @ 2 µg/mL blood;
(2) 0.98 @ 0.05 mg/mL blood;
(3) 0.93 @ 2 µg/mL blood

PRECISION ($\hat{S}_r$): (1) 0.095 (1 µg/mL blood);
(2) 0.056 (0.1 mg/mL blood);
(3) 0.098 (1 µg/mL blood)

APPLICABILITY: MEK and toluene are commonly found in trace amounts in humans working in the paint spray industry. Occasionally, traces of ethanol are also present. This method can be used in screening workers exposed to these central nervous system depressants.

INTERFERENCES: Ethanol concentrations in excess of the stated range may interfere with the MEK peaks at low concentration (< 0.2 µg MEK/mL blood). Ethanol in excess of the stated range also increases blood toluene concentration [1]. When substances other than the analytes are suspected to be present in the blood, record their identities to determine possible interferences [2].

OTHER METHODS: None are known that can determine these constituents simultaneously. Other methods require extraction or distillation for concentration of trace amounts of these chemicals in blood.

REAGENTS:

1. Methyl ethyl ketone, calibration stock solution (3.864 mg/mL). Dilute 1.2 mL MEK (2-butanone) to 250 mL with distilled water. Stable several months at 4 °C.
2. Toluene calibration stock solution (3.81 mg/mL). Dilute to 1.1 mL toluene to 250 mL with acetone. Stable several months.
3. Ethanol calibration stock solution (157.8 mg/mL). Dilute 10 mL absolute ethanol to 50 mL with distilled water. Stable several months at 4 °C.
4. Citric acid.
5. Sodium citrate.
6. Dextrose.
7. Acetone.
8. Isobutanol, 8 µg/mL (aqueous), internal standard.
9. Acidium citricum dextrose solution (ACD). Accurately weigh 4.8 g citric acid, 13.2 g sodium citrate, and 14.7 g dextrose. Dissolve and dilute to 1 L with distilled water.
10. Blood (human), from blood centers or from individuals not exposed to the analytes. * Can be stored at 4 °C for three weeks.

* See Special Precautions.

EQUIPMENT:

1. Tubes, heparin-coated, vacuum, 5-mL.
2. Gas chromatograph with flame-ionization detector, electronic integrator, and column (page 8002-1).
3. Rotator for mixing blood specimens.
4. Vials, screw-cap septum with caps, 7-mL.
5. Septum discs, PTFE-lined.
6. Heater, Dri-block with temperature control (30 to 100 °C) to accommodate the 7-mL size vials.
7. Micropipettes, 100- to 1000-µL.
8. Syringes, gas-tight, 1-mL, with valve.
9. Syringes, tuberculin, disposable, 1-mL, with 21-gauge needle.
10. Volumetric flasks, 50- and 250-mL.

SPECIAL PRECAUTIONS: Samples of blood 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 blood specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided.

SAMPLING:

1. Collect 5 mL venous whole blood in a vacuum tube containing heparin. Invert the tube gently several times to mix.
2. Ship samples in polyfoam packs containing bagged ice or refrigerant.

SAMPLE PREPARATION:

3. Pipet 0.5 mL ACD solution and 0.25 mL isobutanol solution into a 7-mL glass screw-cap vial and mix.
4. Using a tuberculin syringe with a 21-gauge needle, draw 0.25 mL of blood from the vacuum tube and add to the vial.
5. Secure the PTFE-lined septum disc and cap tightly to the vial.
6. Mix the solution very slowly on a rotator for 5 min.

CALIBRATION AND QUALITY CONTROL:

7. Prepare working standard solutions. Pipet 2 mL from each of the calibration stock solutions into separate 200-mL volumetric flasks. Dilute to the mark with deionized water. The resultant concentrations are 38.1 and 38.6 µg/mL for toluene and MEK, respectively; and 1.58 mg/mL for ethanol. Prepare fresh standards every 92 h.
8. Make blood standards at 5 different concentrations ranging from 0 to 8 µg MEK and toluene/mL blood and from 0 to 500 µg ethanol/mL blood. Add aliquots of the working standards to 2 mL blood with a microliter syringe. Prepare fresh blood standards every 92 h and store at 4 °C.
9. Analyze the blood standards (steps 3 through 6 and 12 through 14).
10. Plot the concentrations (µg/mL) vs. the ratio of the peak areas of each analyte to the peak areas of the internal standard (isobutanol) on the same chromatogram to establish the calibration graph.
11. Include a blood control with each run.

MEASUREMENT:

12. Heat the sealed vial in a block heater at 60 °C for 15 min.
13. Remove a 1-mL aliquot of the head space vapor inside the sealed vial with a heated 60 °C gas-tight syringe and inject into the gas chromatograph.
    NOTE: Temperature control of blood and syringe is very important for accurate results. Heat the gas-tight syringe to 60 °C in an oven.
14. Measure the peak areas. Divide the peak area for each analyte by the peak area for the internal standard on the same chromatogram.

CALCULATIONS:

15. Determine the concentrations of MEK, toluene, and ethanol in blood from the calibration graph.

GUIDES TO INTERPRETATION:

1. Blood-alcohol analyses are usually reported in mg/dL or % w/v blood alcohol concentration (BAC). Thus, a BAC may be reported as 300 µg/mL or 30 mg/dL, or 0.03 g/dL, or 0.03% w/v. Legal blood alcohol concentrations are reported in reference [3].
2. Single toluene inhalation exposures of 50 and 100 ppm in air for 3 h produced average blood (human) toluene concentrations of 1.6 and 3.9 ppm, respectively [4]. Another study reported blood toluene levels ranging from 4.1 to 7.3 ppm for exposure to 200 ppm toluene in air [5].
3. The Biological Exposure Index for toluene is 1 mg/L in end-of-shift venous blood [6].

EVALUATION METHOD:

1. Ten spiked replicate blood samples at the blood concentration of 1 µg/mL for MEK and toluene and 10 µg/mL for ethanol were analyzed. The precision data obtained for each analyte were 9.5, 9.8, and 5.6%, respectively.
2. Average recoveries of 10 replicate blood samples for MEK, toluene and ethanol were 90, 93 and 98%, at 3 µg/mL of MEK, 2 µg/mL toluene, respectively, and 0.05 mg/mL of ethanol, respectively, in pooled blood.

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METHOD WRITTEN BY:

William P. Tolos, NIOSH/DBBS.