ACETONE and METHYL ETHYL KETONE in urine 8319

(1) Acetone, CH_3COCH_3	MW: 58.08	CAS: 67-64-1	RTECS: AL3150000
(2) Methyl ethyl ketone, $CH_3COCH_2CH_3$	MW: 72.11	CAS: 78-93-3	RTECS: EL6475000

METHOD: 8319, Issue 1 **EVALUATION: FULL** **Issue 1: 28 October2014**

OSHA & NIOSH: N/A
Because data on exposure limits and guidelines may change over time, NIOSH recommends referring to the following sources for updated limits and guidelines [1-4].

PROPERTIES: (1) bp 56.2 °C; d^{20} 0.789 g/mL
(2) bp 79.6 °C; d^{20} 0.805 g/mL

BIOLOGICAL INDICATOR OF:
Exposure to (1) acetone
(2) methyl ethyl ketone

SYNONYMS: (1) dimethyl ketone; 2-propanone; ketone propane; dimethyl formaldehyde; pyroacetic ether
(2) 2-butanone; MEK; butanone; methyl acetone; butan-2-one; oxobutane

SAMPLING

SPECIMEN: Two urine samples (before and after exposure)

VOLUME: Fill a 20-mL amber VOA vial leaving minimal headspace

SHIPMENT: Ship in an insulated container with bagged refrigerant

SAMPLE STABILITY: Stable at least 30 days at 4 °C [5]

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

ACCURACY

RANGE STUDIED: (1) 2.1 - 606 mg/L [5]
(2) 2.2 - 617 mg/L [5]

ACCURACY: (1) ± 11.5%, (2) ± 15.0%

BIAS: (1) -0.0444, (2) -0.0782

OVERALL PRECISION ($\hat{S}_{rT}$): (1) 0.0468 [5], (2) 0.0507

SAMPLE STABILITY: (1) 101% (Day 30) [5]
(2) 105% (Day 30) [5]

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY, FLAME IONIZATION DETECTOR, HEADSPACE

ANALYTES: Acetone and methyl ethyl ketone

INJECTION VOLUME: 500 µL or timed (see measurement section)

TEMPERATURE
-INJECTION: 180 °C
-DETECTOR: 250 °C
-COLUMN: 40 °C (4 min); 40 to 60 °C @ 3 °C/min; 60 to 220 °C @ 20 °C/min; 220 °C (2 min)

CARRIER GAS: Helium, at 15 psi head pressure

COLUMN: Capillary, fused silica, 6% cyanopropylphenyl, 94% dimethylpolysiloxane, 75 m x 0.53 mm ID, 3.0 µm film thickness

CALIBRATION: Analyte in control urine; 2-pentanone or other appropriate internal standard

ESTIMATED LOD: (1) 0.6 mg/L in pooled urine [5]
(2) 0.6 mg/L in pooled urine [5]

APPLICABILITY: This method can be used in the analysis of acetone and methyl ethyl ketone in urine specimens. These compounds may be found in the urine of individuals exposed to acetone and methyl ethyl ketone [6,7].

INTERFERENCES: Acetone is a metabolite of 2-propanol; MEK is a metabolite of 2-butanol. Exposure to 2-propanol or 2-butanol may result in increased acetone or MEK excretion, respectively [8,9]. Diabetes and fasting also produce elevated urinary acetone levels [10]. Ethanol reduces MEK metabolism and thus increases the MEK concentration in urine [11]. Gender differences and use of hormonal contraceptives have been shown to affect the metabolism and excretion of MEK [12].

OTHER METHODS: There are several commercially-available, direct-reading, dipstick-type tests that are non-specific for ketones in urine. These are often used in hospitals or by diabetic patients. The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) has published a headspace method for a variety of alcohols and ketones in urine and blood [13].

REAGENTS:

1. Pooled control urine collected from unexposed workers*
2. Acetone (≥99%) and MEK (≥99%) stock solution: Prepare by diluting the appropriate amounts of the pure analyte in water.* 750 µL in 10 mL gives ~60 mg/mL of each. Be certain to use the densities and purity factors when calculating the exact concentrations. [5]
3. Internal standard solution: Dilute 80 mg of 2-pentanone (≥99%) in enough water to make 1.0 L (80 mg/L).* Alternatively, 100 µL can be added to 1 L which gives roughly the same concentration.
4. Water, ASTM Type II [14]
5. Helium, purified
6. Hydrogen, prepurified
7. Air, filtered, prepurified

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Bottles, polyethylene screw-top, 125-mL
2. Volatile organic analysis (VOA) vials, 20-mL, amber, polytetrafluoroethylene (PTFE) caps
3. Gas chromatograph, with flame ionization detector (FID), data system and column (p. 8319-1)
4. Headspace autosampler
5. Bagged refrigerant
6. Pipette, 10-mL, plastic or serological
7. Headspace vials, 20-mL, PTFE/Butyl septa, aluminum crimp cap
8. Microliter syringes, 10-µL, 100-µL, 1-mL
9. Volumetric flask, 10-mL

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 [15]. Handle urine samples and urine extracts using proper gloves. Acetone and MEK are highly flammable liquids. All work should be performed in a fume hood since both chemicals are respiratory irritants and may depress the central nervous system at high exposure levels.

SAMPLING:

1. Collect urine in a 125-mL polyethylene bottle or other suitable container. Collect at least two urine samples from each worker. Collect one sample before exposure and one sample after exposure.
   NOTE: It is important to avoid contamination of the urine samples by making sure that samples are collected in a clean area away from the source(s) of exposure and under hygienic conditions (after washing hands.)
2. For each sample, immediately transfer from the 125-mL polyethylene bottle enough urine to fill a 20-mL amber VOA vial such that a minimal headspace is left. Cap the containers tightly. When the VOA vial is inverted no air bubbles should be present. Refrigerate after collection.
3. Collect and pool urine from unexposed workers to be used for controls. Refrigerate after collection.
4. Ship the VOA vials and pooled control urine in a refrigerated, well-insulated container. Store refrigerated upon receipt.

SAMPLE PREPARATION:

5. Allow urine to reach room temperature.
6. Pipet 10.0 mL of urine from the VOA vial into a 20-mL headspace vial.
7. Add 0.5 mL of the internal standard solution.
8. Cap vial immediately.

CALIBRATION AND QUALITY CONTROL:

9. Calibrate daily with at least six working standards covering the concentration range of the method (2 to 600 mg/L).
   NOTE: If the range of concentrations of the samples is known (or expected), the calibration curve range can be adjusted accordingly.
   a. Prepare a diluted stock solution by pipetting 1 mL of the concentrated stock solution into a 10-mL volumetric flask and filling to the mark with pooled urine.
   b. Prepare each working standard by diluting a known amount of the diluted stock solution prepared in Step 9a into enough pooled urine to make a total of 10 mL.
      NOTE: A second, more dilute stock solution in urine can be prepared, if desired, so that the lowest calibration standards are made using more easily-measured spiking volumes.
   c. Prepare at least one pooled urine blank by transferring 10 mL of pooled urine (the same pooled urine used for creating the working standards) into a vial.
   d. Process the 10 mL of each working standard and each pooled urine blank using the same procedure as for the samples (steps 5 through 8).
   e. Analyze the working standards, the pooled urine blanks, and the samples together. FIGURES 1 and 2 show representative chromatograms of blank and fortified urines.
   f. 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 (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. Because humans can endogenously produce both acetone and methyl ethyl ketone, the compounds may be detected 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.
10. Prepare at least two levels of quality control (QC) samples by spiking both analytes in urine. These levels should be at approximately 10-fold the limit of quantitation (LOQ) and 200-fold the LOQ, but can be adjusted to better suit the anticipated levels of the sample set. Unspiked samples of the urine 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.
11. 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 should be taken before more samples are analyzed.

MEASUREMENT:

12. Set gas chromatograph according to manufacturer's recommendations and to conditions given on p. 8319-1.
13. Set headspace autosampler according to manufacturer's recommendations and to the following conditions: (NOTE: different types of headspace samplers may require alternative conditions and some of these might not apply.)

a. Suggested conditions for a balanced-pressure type of headspace system:

Transfer Temp:	129 °C	Withdrawal:	0.2 min
Thermostat Time:	30 min	Needle Temp:	120 °C
GC Cycle Time:	28 min	Sample Temp:	80 °C
Pressurize:	1.0 min	Inject:	0.08 min

b. Suggested conditions for a syringe-injection type of headspace system:

Incubation Temp:	95 °C	Injection volume:	500 µL
Incubation Time:	15 min	Fill speed:	120 µL/sec
Agitation speed:	250 rpm	Delay:	5 sec
Run time:	26 min	Injection speed:	300 µL/sec
Syringe Temp:	95 °C	Delay:	500 msec

14. Measure peak area. Normalize the analyte response by dividing the peak area of the analyte by the peak area of the internal standard on the same chromatogram for each working standard, sample, and pooled urine blank.

CALCULATIONS:

15. Determine both the acetone and MEK concentrations (mg/L) in the urine sample from the calibration graph prepared in Step 9f.

EVALUATION OF METHOD:

This method was evaluated over the ranges of the two analytes specified on p. 8319-1. These ranges represent from 1 x LOQ to 300 x LOQ. Six to seven replicates were analyzed at each level. The average recoveries at the various levels ranged from 94% to 106% for acetone and 85% to 98% for MEK. The LOD and LOQ were determined by preparing and analyzing a series of standards in duplicate with the data fitted to a quadratic curve. The LOD and LOQ were estimated according to Burkart's Method [16]. A long-term storage study was carried out at the 10 x LOQ level. Pooled urine samples spiked with the analytes were stored at 4 °C for 1, 4, 7, 10, 21, or 30 days and then analyzed. All recoveries were nearly 100%. When stored at room temperature, a significant reduction in analyte recovery was observed after 7 days [5].

REFERENCES:

[1] ACGIH [2013]. TLVs® and BEIs® based on the documentation of the Threshold Limit Values for chemical substances and physical agents and Biological Exposure Indices. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists. www.acgih.org [Date accessed: August 2014.]

[2] Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area [2013]. List of MAK and BAT values 2013; Maximum concentrations and biological tolerance values at the workplace. DFG: Deutsche Forschungsgemeinschaft, Wiley-VCH Verlag GmbH & Co. www.dfg.de/en/dfg_profile/publications/senate_commissions/index.html#micro7535782 [Date accessed: August 2014.]

[3] SUVA (Swiss National Accident Insurance Fund) [2013]. Protection de la santé au poste de travail. Valeurs limites d'exposition aux postes de travail. www.suva.ch/english/startseite-en-suva/praevention-en-suva/publications-en-suva.htm [Date accessed: August 2014.]

[4] Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA). www.dguv.de/ifa/Gefahrstoffdatenbanken/GESTIS-Internationale-Grenzwerte-für-chemische-Substanzen-limit-values-for-chemical-agents/index-2.jsp [Date accessed: August 2014.]

[5] Downs CC, Perkins JB [2002]. Back-up data report for acetone and methyl ethyl ketone method development. DataChem Laboratories, Inc. under NIOSH contracts CDC-200-95-2955 and CDC-200-2001-08000.
[6] Pezzagno G, Imbriani M, Ghittori S, Capodaglio E, Huang J [1986]. Urinary elimination of acetone in experimental and occupational exposure. Scand J Work Environ Health *12*:603-608.
[7] Perbellini L, Brugnone F, Mozzo P, Cocheo V, Caretta D [1984]. Methyl ethyl ketone exposure in industrial workers. Uptake and kinetics. Int Arch Occup Environ Health *54*:73-81.
[8] Kawai T, Yasugi T, Horiguchi S, Uchida Y, Iwami O, Iguchi H, Inoue O, Watanabe T, Nakatsuka H, Ikeda M [1990]. Biological monitoring of occupational exposure to isopropyl alcohol vapor by urinalysis for acetone. Int Arch Occup Environ Health *62*:409-413.
[9] Williams RT [1950]. Detoxication mechanisms: the metabolism and detoxication of drugs, toxic substances and other organic compounds. Chapman & Hall Ltd., London p. 66.
[10] Bales JR, Bell JD, Nicholson JK, Sadler PJ [1986]. HNMR Studies of urine during fasting: excretion of ketone bodies and acetylcamitine. Magn Reson Med *3*:849-856.
[11] Liira J, Riihimaki V, Engstrom K [1990]. Effects of ethanol on the kinetics of inhaled methyl ethyl ketone in man. Br J Ind Med *47*:325-330.
[12] Tomicic C, Berode M, Oppliger A, Castella V, Leyvraz F, Praz-Christinaz S-M, Danuser B [2011]. Sex differences in urinary levels of several biological indicators of exposure: A human volunteer study. Toxicol Lett *202*:218-225.
[13] Angerer J, Gündel J [2012]. Alcohols and Ketones [Biomonitoring Methods, 1996]. The MAK Collection for Occupational Health and Safety. pp. 1-33.
[14] ASTM [2011]. ASTM Standard D1193, Standard specification for reagent water. ASTM International, West Conshohocken, PA. DOI 10.1520/D1193-06R11.
[15] CDC [2007]. 2007 Guidelines for isolation precautions: Preventing transmission of infectious agents in healthcare settings. http://www.cdc.gov/hicpac/2007IP/2007isolationprecautions.html. [Date accessed: April 2013.]
[16] Burkart JA [1986]. General procedures for limit of detection calculations in the industrial hygiene chemistry laboratory. Appl Ind Hyg *1*(3):153-155.

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.

FIGURE 1. Representative overlaid chromatograms from a high and low urine standard and urine blanks with and without internal standard (IS), full-scale

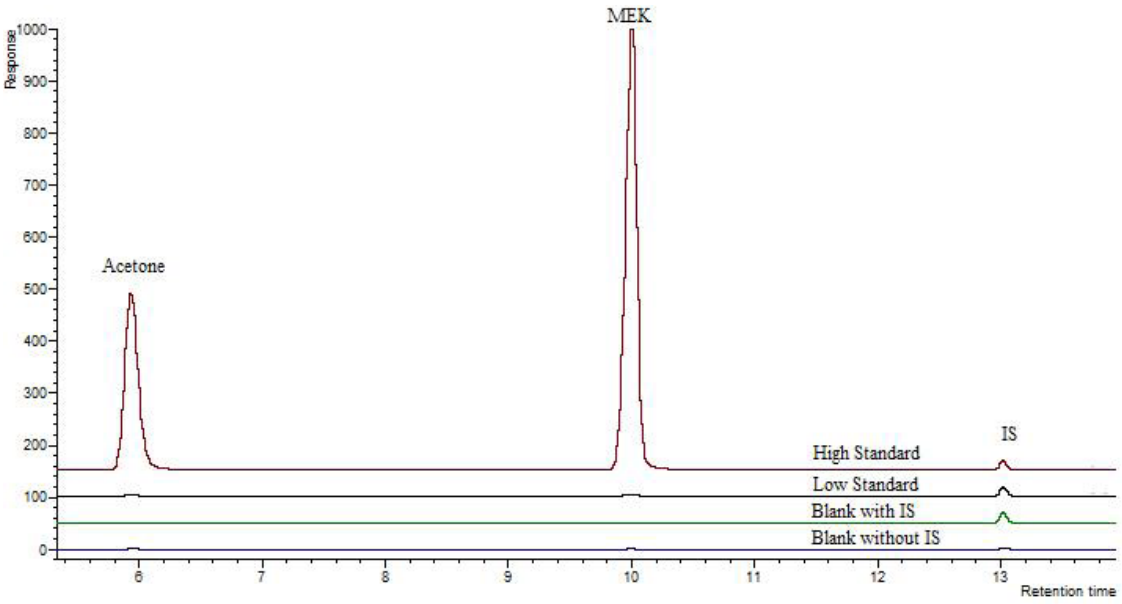

FIGURE 2. Representative overlaid chromatograms from a high and low urine standard and urine blanks with and without internal standard (IS), reduced-scale