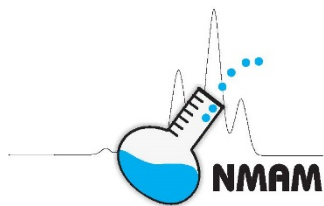

BACKUP DATA REPORT
NIOSH Method No. 8319

Title: Acetone and Methyl Ethyl Ketone in urine

Analyte: Acetone, Methyl Ethyl Ketone

Author/developer: Corey C. Downs, James B. Perkins

Date: 3/7/2014

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ACETONE AND METHYL ETHYL KETONE IN URINE

NMAM 8319, ISSUE 1

BACK-UP DATA REPORT

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ACETONE and METHYL ETHYL KETONE in urine:

Back-up Data Report

INTRODUCTION AND BACKGROUND

Both acetone and methyl ethyl ketone (MEK) are utilized in a wide variety of industrial processes. They are present as solvents in many commercial products such as paints, inks, adhesives, thinners, and resins. The ubiquitous use of these chemicals in industry generates a large potential for excessive occupational exposure.

Symptoms of acetone and MEK exposure are similar. Lower concentrations primarily cause irritation of mucous membranes. Higher levels non-specifically depress the central nervous system and are associated with headaches, drowsiness, dizziness, confusion, and even unconsciousness. The onset of the effects varies broadly from person to person and seems to be influenced by prior exposure.

Individuals exposed to acetone or MEK will excrete a portion of the chemical in their urine. Several studies indicated a significant correlation between environmental exposure and urinary excretion levels for both acetone and MEK; therefore, monitoring occupational exposure to these solvents can be achieved through urinalysis [1-4]. Acetone and MEK pass into the urine via simple renal diffusion; their urinary concentrations should not be corrected using creatinine or specific gravity measurements and will be unaffected by renal disease. The US Environmental Protection Agency has published toxicological reviews of both compounds [5, 6].

This report evaluates a method for simultaneously quantifying acetone and MEK concentrations in urine samples utilizing a gas chromatograph equipped with a flame ionization detector (FID). This method employs a headspace technique in which the air above a sample is injected into the system, rather than injecting the actual sample or an extract of the sample. A method based on a headspace technique seems ideal in this situation because the volatile nature of the analytes allows them to easily enter the headspace upon heating of the sample. It also eliminates the need for extra sample extraction steps and prevents injecting relatively dirty biological matrices into the instrument, where contamination and wear may occur.

Caution must be used in the interpretation of results from this analysis, as other urinary sources are documented for each analyte, which could lead to an overestimation of the exposure

levels. All humans endogenously produce acetone as a natural part of daily metabolism. This method is sensitive enough to detect endogenous concentrations of acetone. Diabetics will have significantly higher concentrations of acetone in their urine. Fasting may also elevate acetone urinary concentrations [7]. Acetone is a metabolite of 2-propanol, consequently 2-propanol exposure can lead to increased acetone concentrations in urine [8]. MEK is not usually endogenously produced by humans and is normally only found in persons occupationally exposed to the solvent. However, 2-butanol is metabolized to MEK and may interfere with monitoring MEK exposure [9]. Simultaneous exposure to ethanol was shown to reduce MEK metabolism and thus increase the MEK concentration in urine; consumption of alcoholic beverages may cause an increase in MEK urinary concentrations [10].

The Biological Exposure Indices (BEI) Committee recommends a BEI in urine of 2 μg/mL for MEK and 50 μg/mL for acetone [11].

NOTE: Proper safety precautions should always be taken when dealing with any chemical but especially when working with biological fluids such as urine. Manipulating biological samples poses a serious health risk because of the potential transmittance of infectious diseases including hepatitis and HIV. Lab coats, goggles, and gloves must be worn at all times and standard precautions should be followed [12]. Work should be performed in an isolated hood where possible. All waste is required by law to be disposed of in a properly labeled, autoclavable container.

REAGENTS AND MATERIALS

Presented in Table 1 is the list of reagents and solvents used for this method and its evaluation. 2-Pentanone was selected for use as an internal standard to normalize the values of acetone and MEK determined in the urine samples.

TABLE 1. LIST OF CHEMICALS

Chemical	Vendor	CAS #	Purity	Lot #
Acetone	Aldrich	67-64-1	99%	DO 033337 DO
Methyl Ethyl Ketone	Aldrich	78-93-3	99.5%	LI 03563 KI
2-Pentanone	Aldrich	107-87-9	97%	02009HT
Water		7732-18-5	ASTM Type II	--

Water was used as the solvent while preparing the primary stock and internal standard solutions. Urine used in the study for standards and test samples was collected from volunteer employees at DataChem Laboratories in Salt Lake City. The urine was collected and pooled as needed. Once pooled, it was stored at 4 °C in 1-L polyethylene screw-top bottles.

Preparation of Primary Stock and Internal Standard Solutions

To make the primary stock solution, a 10-mL volumetric flask was filled partially with water. With a microliter syringe, a specific, measured volume of each analyte was added to the flask. The 10-mL volumetric flask was brought to volume with water, mixed, and the solution was transferred to a 13 X 100 mm glass culture tube with a Teflon-lined cap. The solution was stored at 4 °C in the dark until needed. The concentration of each analyte in the stock solution was calculated using the volume of analyte added, its density, its purity factor, and the total dilution volume. Using acetone as an example:

$$770\,\mu L \times \left(\frac{0.788 \cdot mg}{\mu L}\right) \times (0.999) \times \left(\frac{1}{10 \cdot mL}\right) = 60.6 \text{ mg/mL.}$$

The internal standard solution was prepared in a similar manner by adding 2-pentanone to enough water to fill a 1-L volumetric flask. The target concentration was approximately 80 μg of 2-pentanone/mL of water. For the experiments in this evaluation, the actual internal standard concentration was 78.5 μg/mL calculated as follows:

$$100\,\mu L \times \left(\frac{0.8095 \cdot mg}{\mu L}\right) \times (0.97) \times \left(\frac{1}{1 \cdot L}\right) = 78.5 \text{ mg/L} = 78.5\ \mu\text{g/mL.}$$

This concentration of internal standard was employed because it provided good peak shape, peak area, and reproducibility for the internal standard.

Sample Preparation and Instrument Conditions

Sample Preparation

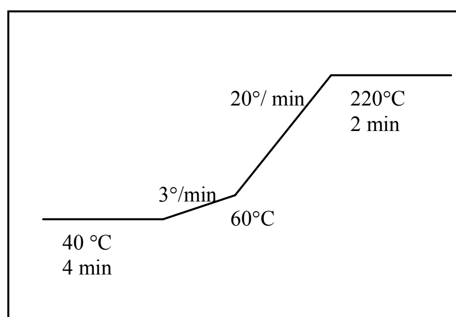
For each study, the samples and standards were always prepared in the same manner. Exactly 10.0 mL of urine was transferred into a 20-mL Perkin Elmer HS-40 headspace vial. Half a milliliter of the internal standard solution was added to the vial before sealing the vial with an aluminum crimp-cap and PTFE/Butyl septum. Each vial was then lightly mixed and analyzed. If spiked urine and samples were not going to be transferred to headspace vials and analyzed immediately, they should be stored at 4 °C in tightly-sealed vials with minimal headspace.

Samples shipped from the field should also be sent refrigerated in containers having as little headspace as possible.

Instrument Conditions

All of the samples and standards were run on the same system with the same set of conditions. The system consisted of a Perkin Elmer Autosystem gas chromatograph equipped with an FID and a Perkin Elmer HS-40 headspace autosampler. The column was a fused silica capillary column (DB-624, 75 m X 0.53 mm I.D., 3.0 µm film). Figure 1 shows the temperature program that was utilized. The head pressure was maintained at 15 psi.

FIGURE 1. TEMPERATURE PROGRAM



The carrier gas consisted of pre-purified helium and the FID was supplied with pre-purified hydrogen and filtered air. The injector and detector temperatures were 180 °C and 250 °C respectively. The injection conditions were splitless. The headspace autosampler was set to the following conditions:

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

Table 2 shows the typical retention times of the analytes given these conditions.

TABLE 2. ANALYTE RETENTION TIMES

Analyte	Retention Time (min)
Acetone	5.46
MEK	9.43
2-Pentanone	13.20

The final temperature (220 °C) was maintained until the column appeared to be clean in order to prevent any carryover into the next sample injection. Conditions used for a syringe-injection type of headspace system can be found in the Appendix.

Calibration

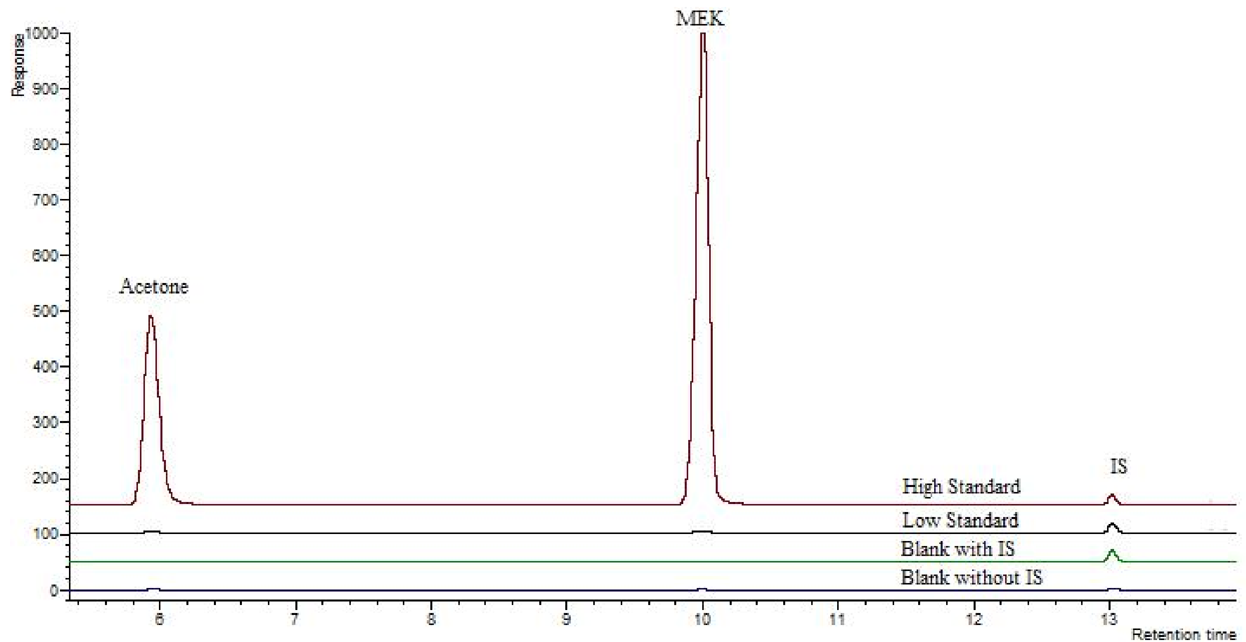
To quantify the amount of acetone and MEK present in samples, a calibration curve was constructed daily with at least six working standards covering the anticipated concentration range of the samples. The working standards were prepared by diluting known amounts of the acetone/MEK stock solution into enough pooled urine to make a total of 10.0 mL for each standard. Along with the working standards, at least one pooled urine blank was prepared by transferring 10.0 mL of pooled urine (the same pooled urine used for creating the working standards) into a headspace vial. The 10 mL of each working standard and pooled urine blank were then processed using the same procedure as listed previously.

After analyzing the samples, a calibration graph was generated for each analyte 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. concentration of analyte on the x-axis. A linear or quadratic model was utilized in processing the working standard data, depending on which model provided a better fit to the data. Because humans endogenously produce acetone, detectable amounts of acetone were often found in the pooled urine blanks. Before plotting the calibration graph, it was often necessary to subtract the normalized analyte response of the pooled urine blank from the normalized analyte response of each working standard. The normalized analyte response was then calculated for each sample and the corresponding acetone and MEK concentrations were read from the x-axis of the calibration curves.

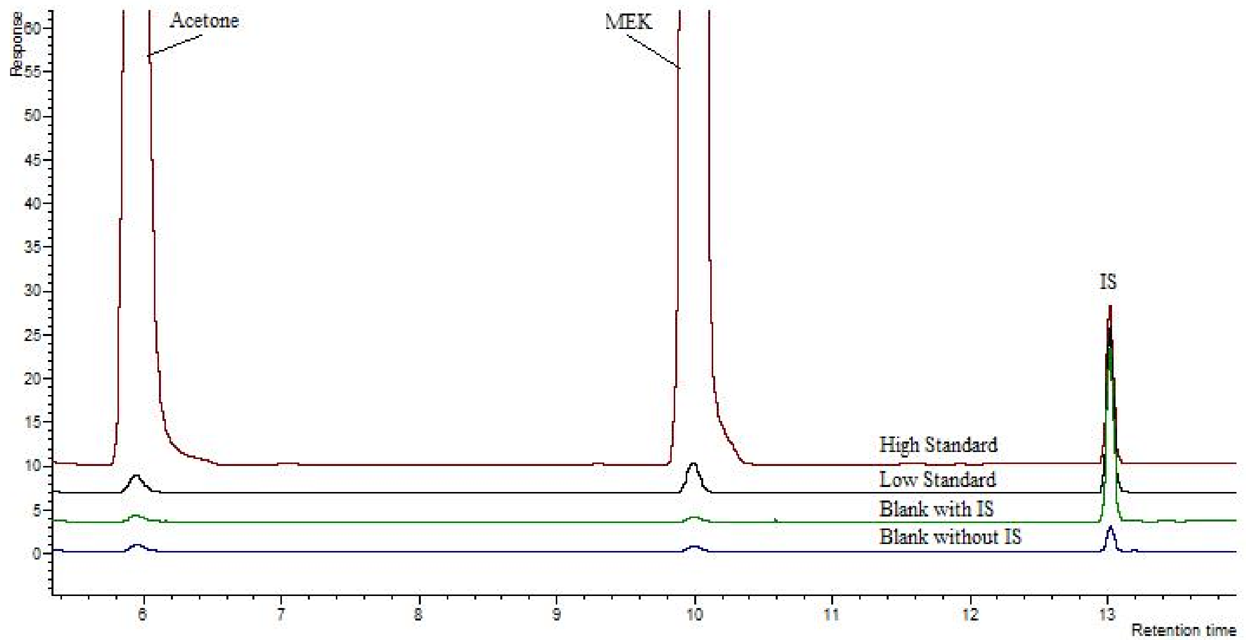
Chromatogram Analysis

Representative overlaid chromatograms of a high and low urine standard and of blank urine with and without internal standard when the previously described conditions are employed are shown in both full- and reduced-scales in Figure 2. 2-Pentanone was chosen as the internal standard because of its availability and because its retention time positioned it in a relatively interference-free portion of the chromatogram of the urine sample.

FIGURE 2. REPRESENTATIVE CHROMATOGRAMS



The upper set of chromatograms is shown in full-scale while the lower set of chromatograms is shown in reduced-scale to allow more details of the baseline to be evident.



It may appear that the initial temperature program ramp begins too slowly and that higher initial temperatures and ramps would decrease the analysis time. Although this may be the case, it is not recommended. Field samples tended to be quite variable. A more aggressive temperature program caused overlapping and interfering peaks in some samples. In particular, acetone would

often co-elute with other compounds when higher initial temperatures were employed. These chromatograms were acquired during the User Check experiments and thus show slightly different retention times than those shown in Table 2.

LIMIT OF DETECTION AND QUANTITATION STUDY

This study was to determine the limit of detection (LOD) of the method based upon pooled control urine spiked with acetone and MEK. The limit of quantitation (LOQ) for both analytes was then calculated as 10/3 times the LOD. The resulting LOQ was then used to determine the target concentrations for all subsequent steps in the method evaluation.

A range of standards for analysis was prepared in duplicate by diluting the primary stock solution (described in Reagents and Materials section) and spiking the appropriate volume into 10 mL of pooled control urine. Table 3 lists the range of concentrations used during the study. The samples were then prepared and analyzed as described in the Sample Preparation and Instrument Conditions section. The LOD and LOQ were estimated by fitting the data to a quadratic curve followed by applying Burkart's Method to the data [13].

TABLE 3. LOD/LOQ STANDARDS

Standard	Acetone (µg/mL)	MEK (µg/mL)
$1	303	308
$2	212	216
$3	121	123
$4	30.3	30.8
$5	21.2	21.6
$6	12.1	12.3
$7	3.03	3.08
$8	1.21	1.23
$9	0.606	0.617
$10	0.303	0.308
$11	0.182	0.185

The correlation coefficient for both analytes was 0.999 for the quadratic curve. The data provided LOD estimates of 0.6 and 0.5 µg/mL for acetone and MEK, respectively. This equates to LOQs of 2.0 and 1.7 µg/mL for the two analytes. Standard 11 was difficult to distinguish from the base-line noise.

This experiment was repeated three different times on three different days using slightly different concentrations of standards to determine if the LOD was consistently achievable. The LODs ranged from 0.3 to 0.8 µg/mL for acetone and 0.1 to 0.7 µg/mL for MEK. Although lower LODs were occasionally obtained, 0.6 µg/mL was chosen for each analyte as the method's LOD because it was consistently obtainable.

LONG-TERM STABILITY STUDY

This experiment was designed to assess the stability of acetone and MEK in urine under various conditions of storage. The study covered a period of 30 days with samples stored both at room temperature (24 °C) and refrigerated (4 °C). The study was performed using pooled urine for both samples and standards. For samples to be considered stable, they must have a loss of less than 10% over a period of seven days [14]. Once at the laboratory, the analysis might be further delayed for various reasons; therefore, the study was extended for 30 days.

A 2-L volume of pooled control urine was prepared in a volumetric flask so that it contained 21.1 and 21.5 µg/mL of acetone and MEK, respectively (approximately 10 x LOQ). The spiked urine was immediately split into 30 samples by pouring it into 20-mL amber VOA vials. The urine was added until the vial was completely full. The vials were capped with Teflon PTFE septa and screw-cap tops. When inverted, no air bubbles were visible on the bottom of the containers. Eight blank samples were prepared in the same manner using pooled control urine that was not spiked with the analytes of interest. The samples were then either left at room temperature or stored in a refrigerator at 4 °C. On each day, the designated samples plus one blank were analyzed. With each sample set, a fresh series of standards was prepared and analyzed each day to produce a calibration curve. Table 4 shows the schedule for the analysis of the samples stored at room temperature (24 °C), the length of storage and the results for each set of samples.

TABLE 4. LONG TERM STABILITY STUDY AT ROOM TEMPERATURE (24 °C)

Storage Time	Sample #	Storage Temperature	Target Value µg/mL (Acetone)	Amount Recovered µg/mL (Acetone)	Recovery [%] (Acetone)	Target Value µg/mL (MEK)	Amount Recovered µg/mL (MEK)	Recovery [%] (MEK)
1 Day	1	24 °C	21.1	21.1	100.0	21.5	21.4	99.5
	2	24 °C	21.1	21.5	101.9	21.5	21.3	99.1
	3	24 °C	21.1	21.6	102.4	21.5	22.2	103.3
	4	24 °C	21.1	21.3	100.9	21.5	22.8	106.0
	5	24 °C	21.1	20.4	96.7	21.5	21.3	99.1
	6	24 °C	21.1	22.3	105.7	21.5	22.9	106.5
7 Days	1	24 °C	21.1	17.9	84.8	21.5	19.1	88.8
	2	24 °C	21.1	15.1	71.6	21.5	17.6	81.9
	3	24 °C	21.1	17.2	81.5	21.5	19.9	92.6
	4	24 °C	21.1	18.7	88.6	21.5	20.9	97.2
	5	24 °C	21.1	16.0	75.8	21.5	18.3	85.1
	6	24 °C	21.1	15.1	71.6	21.5	18.3	85.1
Average			21.1	19.0	90.1	21.5	20.5	95.3
Std. Dev.			0	2.69	12.7	0	1.82	8.5

The samples are clearly stable for 24 hours at room temperature. However, after seven days at room temperature, analyte loss was significant. Samples should, therefore, always be shipped and stored at 4 °C or cooler.

To determine the stability of the urine samples for longer terms of storage, up to 30 days, an experiment was performed as per the recommendations from the "NIOSH Guidelines for Air Sampling and Method Development and Evaluation" [14]. The 20-mL VOA vial urine samples were divided into one group of 6 and four groups of 3. Since the data from the one-day storage at room temperature were already obtained from the previous experiment, it was not deemed necessary to repeat the experiment a second time. The eighteen samples were refrigerated at 4 °C. The group of 6 samples was analyzed after 7 days. The remaining four groups of 3 samples each were analyzed after 10, 14, 21, and 30 days. Results from the refrigerated study are presented in Table 5.

TABLE 5. LONG TERM STABILITY STUDY AT 4 °C

Storage Time	Sample #	Storage Temperature	Target Value µg/mL (Acetone)	Amount Recovered µg/mL (Acetone)	Recovery [%] (Acetone)	Target Value µg/mL (MEK)	Amount Recovered µg/mL (MEK)	Recovery [%] (MEK)
7 Days	1	4 °C	21.1	21.4	101.4	21.5	21.9	101.9
	2	4 °C	21.1	21.4	101.4	21.5	21.0	97.7
	3	4 °C	21.1	20.6	97.6	21.5	20.8	96.7
	4	4 °C	21.1	21.1	100.0	21.5	20.1	93.5
	5	4 °C	21.1	21.8	103.3	21.5	21.1	98.1
	6	4 °C	21.1	21.9	103.8	21.5	20.0	93.0
10 Days	1	4 °C	21.1	21.5	101.9	21.5	21.1	98.1
	2	4 °C	21.1	21.2	100.5	21.5	20.9	97.2
	3	4 °C	21.1	20.2	95.7	21.5	20.6	95.8
14 Days	1	4 °C	21.1	20.1	95.3	21.5	21.6	100.5
	2	4 °C	21.1	19.9	94.3	21.5	21.5	100.0
	3	4 °C	21.1	22.8	108.1	21.5	22.9	106.5
21 Days	1	4 °C	21.1	21.5	101.9	21.5	21.7	100.9
	2	4 °C	21.1	21.8	103.3	21.5	22.2	103.3
	3	4 °C	21.1	21.6	102.4	21.5	21.9	101.9
30 Days	1	4 °C	21.1	21.5	101.9	21.5	22.4	104.2
	2	4 °C	21.1	21.1	100.0	21.5	22.6	105.1
	3	4 °C	21.1	21.6	102.4	21.5	22.5	104.7
Average			21.1	21.3	100.8	21.5	21.5	99.9
Std. Dev.			0	0.71	3.4	0	0.85	4.0

The results from the long-term stability study show that acetone and MEK are stable in urine for at least 30 days as long as they are kept at 4 °C with no headspace in the sample container.

PRECISION, ACCURACY, AND BIAS STUDY

A study was performed to determine the precision, accuracy, and bias of the method using pooled control urine. The bias for a method should be less than or equal to 10%. The coefficient of variation (CV) should be less than or equal to 0.1. When these conditions are met, the method is deemed unbiased and accurate with 95% probability to within ±25% of the actual concentrations [14].

During the study, six concentrations ranging from 1 x LOQ (2.0 µg/mL) to 300 x LOQ (600 µg/mL) were studied. For each sample, a volume of 10 mL of pooled urine was used. Seven samples at each level (a total of 42 samples) were spiked with an acetone and MEK solution (described in Reagents and Materials section) to produce the desired concentrations. A sample blank was also included at each level. The samples were then prepared and analyzed according to the method. Table 6 displays the average recoveries of the analytes over the ranges studied.

TABLE 6. AVERAGE RECOVERIES OVER VARIOUS LEVELS

Level (xLOQ)	Rough Target (µg/mL)	Actual Spike (µg/mL)		Average Recovery (%)		CV	
Both	Both	Acetone	MEK	Acetone	MEK	Acetone	MEK
1	2	2.12	2.16	106	98.0	0.110	0.076
3	6	6.06	6.17	92.2	85.5	0.043	0.066
10	20	21.2	21.6	94.9	89.3	0.032	0.044
30	60	60.6	61.7	96.8	96.8	0.019	0.018
100	200	212	216	99.0	98.1	0.062	0.056
300	600	606	617	94.1	89.4	0.062	0.058

The data were processed to determine the coefficient of variation (CV) at each concentration level and whether these levels could be pooled using Barlett's test of homogeneity [14]. The pooled coefficients of variation were then used for calculating the overall precision and accuracy of the method as presented in Table 7. The 1 x LOQ level was not included in the calculations presented in Table 7.

TABLE 7. PRECISION, BIAS, AND ACCURACY

Analyte	Range (µg/mL)	Accuracy (%)	Bias		Precision
			Average	Range	Overall ($\hat{S}_{rT}$)
Acetone	6.1 to 606	11.5	-0.0444	-0.0780 to -0.0103	0.0468
MEK	6.2 to 617	15.0	-0.0782	-0.1453 to -0.0316	0.0507

SUMMARY

All data obtained during the method development met all NIOSH criteria for precision, bias, and accuracy in all studies performed [14]. The method proved to be rugged and adaptable to human urine samples.

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APPENDIX

User Check Conditions

There are three types of headspace autosamplers in common use: syringe injection, balanced pressure, and pressurized loop. The type used in the development of this method and the conditions found herein are for a balanced pressure system. The secondary laboratory validation, known as a User Check, was performed on a syringe injection type system. The chromatographic conditions remain the same, but different autosampler parameters are employed in the different systems. The autosampler conditions utilized in the User Check are as follows:

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

User Check data for acetone and MEK from a first trial is shown in the following tables.

User Check Results

Trial 1

Acetone (concentrations in mg/L)

Target conc	Analyzed conc	Recovery		Ave recovery	Std. Dev.	RSD
0	ND					
0	0.624					
0	ND					
0	1.02					
0	ND					
5.03	4.71	93.6				
5.03	4.70	93.4				
5.03	5.29	105.2				
5.03	5.49	109.2				
5.03	4.95	98.4		100.0	7.01	7.01
10.06	13.60	135.2				
10.06	11.80	117.3				
10.06	11.70	116.3				
10.06	12.90	128.2				
10.06	15.40	153.1		130.0	15.1	11.6
100.6	98.2	97.6				
100.6	101.0	100.4				
100.6	99.6	99.0				
100.6	102.0	101.4				
100.6	100.0	99.4		99.6	1.43	1.44
503	494.0	98.2				
503	478.0	95.0				
503	478.0	95.0				
503	520.0	103.4				
503	555.0	110.3		100.4	6.52	6.50
Overall average		107.5				
Std. Dev.		15.7				
RSD		14.6				

ND = not detected

Methyl ethyl ketone (concentrations in mg/L)

Target + blank conc	Analyzed conc	Recovery	Ave blank conc	Ave recovery	Std. Dev.	RSD
0	0.553					
0	1.69					
0	1.22					
0	2.02					
0	0.974		1.29			
6.26	5.70	91.1				
6.26	5.78	92.3				
6.26	6.18	98.7				
6.26	6.15	98.2				
6.26	6.04	96.5		95.4	3.49	3.65
11.23	14.0	124.7				
11.23	12.3	109.5				
11.23	12.6	112.2				
11.23	13.3	118.4				
11.23	15.0	133.6		119.7	9.73	8.13
100.69	98.6	97.9				
100.69	101.0	100.3				
100.69	99.8	99.1				
100.69	101.0	100.3				
100.69	104.0	103.3		100.2	1.99	1.99
498.29	491.0	98.5				
498.29	480.0	96.3				
498.29	498.0	99.9				
498.29	521.0	104.6				
498.29	532.0	106.8		101.2	4.32	4.27
Overall average		104.1				
Std. Dev.		10.8				
RSD		10.4				

Methyl ethyl ketone was found in the blank urine and only the blank-corrected values are shown. As can be seen from the data, three of the four levels for each compound showed high accuracy and precision while the ~10 mg/L level gave values that were significantly higher and more imprecise than expected. Since no instrumental difficulties were noticed and since levels both above and below this one in concentration gave acceptable data, an error in the preparation of the samples was suspected. A second set of samples was prepared and the results are shown below.

Trial 2

Acetone (concentrations in mg/L)

Target conc	Analyzed conc	Recovery		Ave recovery	Std. Dev.	RSD
0	0.08					
0	0.246					
0	0.272					
0	0.214					
0	0.209					
4.97	4.76	95.8				
4.97	5.01	100.8				
4.97	5.20	104.6				
4.97	5.66	113.9				
4.97	5.64	113.5		105.7	7.92	7.50
9.94	9.92	99.8				
9.94	10.60	106.6				
9.94	10.50	105.6				
9.94	10.50	105.6				
9.94	11.30	113.7		106.3	4.94	4.65
99.4	97.8	98.4				
99.4	102.0	102.6				
99.4	103.0	103.6				
99.4	104.0	104.6				
99.4	99.5	100.1		101.9	2.57	2.53
497	472.0	95.0				
497	499.0	100.4				
497	507.0	102.0				
497	537.0	108.1				
497	528.0	106.2		102.3	5.15	5.03
Overall average		104.1				
Std. Dev.		5.42				
RSD		5.21				

Methyl ethyl ketone (concentrations in mg/L)

Target conc	Analyzed conc	Recovery		Ave recovery	Std. Dev.	RSD
0	ND					
0	ND					
0	ND					
0	ND					
0	ND					
5.07	5.12	101.0				
5.07	5.24	103.4				
5.07	5.26	103.8				
5.07	5.39	106.3				
5.07	5.30	104.5		103.8	1.93	1.86
10.1	10.3	102.0				
10.1	10.5	104.0				
10.1	10.6	105.0				
10.1	10.5	104.0				
10.1	10.5	104.0		103.8	1.08	1.05
101.4	100.0	98.6				
101.4	104.0	102.6				
101.4	103.0	101.6				
101.4	105.0	103.6				
101.4	103.0	101.6		101.6	1.84	1.82
507.1	492.0	97.0				
507.1	507.0	100.0				
507.1	510.0	100.6				
507.1	527.0	103.9				
507.1	522.0	102.9		100.9	2.71	2.68
Overall average		102.5				
Std. Dev.		2.25				
RSD		2.19				

ND = not detected

Methyl ethyl ketone was not found in the blank urine used in this trial. All concentration levels of both compounds fall well within acceptable limits of accuracy and precision. This lends further credence to the theory that the out of range level found in Trial 1 was a sample preparation problem. Below are summary tables combining data from both sets of samples with the suspect level dropped from the calculations.

Acetone

Concentration (mg/L)	Average recovery (%)	Std. Dev.	RSD
5	102.8	7.68	7.46
10	106.3	4.94	4.65
100	100.7	2.31	2.29
500	101.4	5.63	5.56
All combined	102.3	5.63	5.50

Methyl ethyl ketone

Concentration (mg/L)	Average recovery (%)	Std. Dev.	RSD
5	99.6	5.17	5.19
10	103.8	1.08	1.05
100	100.9	1.95	1.94
500	101.1	3.40	3.37
All combined	101.0	3.61	3.57