8.1 ALCOHOL PROCEDURE (PURCHASED OR IN-HOUSE PREPARED CALIBRATORS)

8.1.1 Purpose

To qualitatively and/or quantitatively identify ethanol, methanol, acetone and isopropanol in submitted evidence by instrumental analysis with headspace gas chromatography/flame ionization (HS-GC/FID) and headspace gas chromatography/mass spectrometry (HS-GC/MS). For the analysis of additional volatile compounds (e.g., toluene), see Additional Volatile Compound Procedure (Section 8.7).

8.1.2 Specimen Requirements

Acceptable samples for this analysis include blood, urine, vitreous humor, and other aqueous liquids. For additional samples see Alternative Matrices (section 6.6).

8.1.3 Apparatus and Equipment

Volumetric pipettes and disposable tips
Assorted volumetric glassware
Thermometer (NIST Traceable)
Test tube rotator
20 mm headspace vials (size 20 mL or 22 mL)
Crimp caps with septa
20 mm crimper
Hamilton diluter/dispenser
HS-GC/FID, HS-GC/MS, ChemStation or MassHunter software, compatible computer (and printer, if necessary)

Additional Equipment necessary if preparing in-house calibrators and/or controls
Burette (NIST Traceable)

8.1.4 Reagents and Standards

Ethanol reference standard (NIST traceable)
0.01667 v/v% n-Propanol (internal standard)
Purchased or prepared calibrators and controls
Water (H_2O)

Additional items necessary if preparing in-house calibrators and/or controls:
Ethanol (CH_3CH_2OH)
Acetone ($(CH_3)_2CO$)
Isopropanol ($(CH_3)_2CHOH$)
Methanol (CH_3OH)

8.1.5 Standard Preparation
The following is an example of how to prepare the internal standard used in this procedure.

n-Propanol Reference Standard Solution [0.01667 v/v%] (Internal Standard)
Add 166.7 µL of n-propanol and dilute to 1000 mL with H_2O.

Calibrators and Controls
Reference Standards containing methanol, isopropanol, acetone, and ethanol at the following concentrations will either be purchased or prepared in house. Purchased calibrator and control material shall be stored in tightly sealed containers, with minimum headspace in the refrigerator and will expire one month after opening or at the manufacturer's expiration date, whichever comes first.

Calibrators:

- (0.1 mg/ml = 0.01 gm%)
- (0.3 mg/ml = 0.03 gm%)
- (0.4 mg/ml = 0.04 gm%)
- (0.5 mg/ml = 0.05 gm%)
- (1.0 mg/ml = 0.10 gm%)
- (2.0 mg/ml = 0.20 gm%)
- (5.0 mg/ml = 0.50 gm%)

Controls:

- Multicomponent Alcohol Mix (1000 ug/ml = 0.10 gm%)
- Ethanol reference standard (0.08 gm% NIST traceable)

Preparation of In-House Preparation Reference Materials

MIAE Stock Reference Standard Solution [1 gm%] (A & B)
The volumes listed for methanol, acetone and isopropanol are based on the densities of these liquids at room temperature. The volume of ethanol shall be determined based on the actual temperature of the liquid when it is prepared. The example listed below for ethanol uses the density at 22°C.

To correct for the density of ethanol at the temperature at which it is prepared, refer to the *Density of Alcohol* table below. The prep temperature used for the density calculation must be recorded on the Ethanol Stock Prep Worksheet (See Appendix) or in the appropriate log.

Example: Add 12.69 mL of 99.5+% ethanol, 12.62 mL of methanol (density 0.792 g/cm^3), 12.73 mL of isopropanol (0.785 g/cm^3), and 12.62 mL of acetone (0.792 g/cm^3) and dilute to 1000 mL with H_2O.

Density of Alcohol
Density of Ethyl Alcohol in Grams Per Cubic Centimeter
Computed from Mendeleeff's Formula
(Selected from Smithsonian Tables)

Temp °C	0	1	2	3	4	5	6	7	8	9
0	0.80625	0.80541	0.80457	0.80374	0.80290	0.80207	0.80123	0.80039	0.79956	0.79872
10	0.79788	0.79704	0.79620	0.79535	0.79451	0.79367	0.79283	0.79198	0.79114	0.79029
20	0.78945	0.78860	0.78775	0.78691	0.78606	0.78522	0.78437	0.78352	0.78267	0.78182
30	0.78097	0.78012	0.77927	0.77841	0.77756	0.77671	0.77585	0.77500	0.77414	0.77329

Volatile Working Reference Calibrator/Control Standard Solutions
To make the volatile working reference calibrator/control standard solutions, add the following amount of stock reference standard solution for each compound and dilute to a final volume of 100 mL with H_2O.

SOLUTION gm%	AMOUNT USED	CONCENTRATION	TOTAL VOLUME
0.01	1 mL	MIAE Stock Soln.	100 mL
0.03	3 mL	MIAE Stock Soln.	100 mL
0.04	4 mL	MIAE Stock Soln.	100 mL
0.05	5 mL	MIAE Stock Soln.	100 mL
0.10	10 mL	MIAE Stock Soln.	100 mL
0.20	20 mL	MIAE Stock Soln.	100 mL
0.50	50 mL	MIAE Stock Soln.	100 mL

8.1.6 Sample Preparation for Alcoholic Beverages (Proofs)

Alcohol beverage proof dilutions have been assessed and fit for use at 1:50 and 1:100.

1. Record the temperature of the alcoholic beverage using a NIST traceable thermometer. Document the serial number of the thermometer and the temperature of the alcoholic beverage in case notes.
2. Dilute the alcohol beverage in a volumetric flask as follows:
    a. 1 mL of alcoholic beverage sample filled to a total of 50 mL of water
    b. 1 mL of alcoholic beverage sample filled to a total of 100 mL of water
3. Sample is now ready to proceed with sampling in 8.1.7. See 8.1.8.4 for instructions on calculating v/v% based on ethanol density at temperature previously recorded.

8.1.7 Procedure

1. Allow all reference standards and case samples to equilibrate to room temperature.
2. Mix sample specimens thoroughly on a rotator to ensure homogeneity.
3. Label, check, and load/unload all samples in accordance with the "Sample Pipetting Check List" (see Appendix section).
4. Pipette 100 μL of corresponding case sample, calibrator, positive control, or negative control into the appropriately labeled 20 mm headspace vial.
5. Pipette 600 μL of internal standard into each sample to make a final concentration of 0.1 v/v%.
   Note 1: Smaller sample volumes may be analyzed on a case-by-case basis. The total volume of liquid in the headspace vial must always be equal to 700 μL (e.g., 50 μL sample + 50 μL H_2O + 600 μL internal standard = 700 μL total volume).
   Note 2: Step 5 may precede step 4 based on scientist preference.
6. Seal vial with crimp cap.
7. Analyze and quantitate the samples by HS-GC/FID and confirm by HS-GC/MS (full scan mode).
8. All sample runs will be reassembled in reverse case sample order and steps 1-7 above will be repeated. If not possible, the case sample shall follow a different sample in both duplicate batch runs (e.g., a single case sample moved to a different batch of cases).
   Note 1: Rotation of samples shall not be necessary when it has not been longer than 24 hours since the initial sample rotation.
   Note 2: All samples will be run in duplicate whenever possible; however, in cases where the result is negative and the sample is limited and/or needed for drug analysis, the scientist and/or Toxicology Unit Supervisor/Technical Leader should closely examine the case to determine if only one analysis is appropriate. After careful review, if it is determined that only one analysis is needed, documentation shall be made and included in the case file.
   Note 3: Validation testing showed that samples that may not contain sufficient volume for duplicate testing may be diluted 1:2 for analysis. The dilution will raise the LOD for that sample.
   Note 4: Samples are not always analyzed immediately after pipetting due to unforeseen delays. For example, the instrument may lose communication, or inadvertently shut down during a batch run. Pipetted and sealed samples are stable and fit for use up to 24 hours at room temperature or refrigerated. Reinjection of sample is permitted up to 24 hours.

8.1.8 Reporting

Results can be reported if the following criteria are met:

8.1.8.1 Qualitative

8.1.8.1.1 Retention times of target compounds identified are within ±1% of those of a calibrator or control standard of similar concentration on GC/FID unless otherwise noted in the case file.

8.1.8.1.2 Mass spectrums of drugs identified are consistent with those of analyzed reference standards.

8.1.8.2 Quantitative

8.1.8.2.1 All the qualitative result criteria above must be met.

8.1.8.2.2 Sample ethanol concentrations below 0.01 gm% (the lowest calibration point) shall be reported as “negative”. Other volatiles shall be reported when determined to be present at or above 0.01 gm%.

8.1.8.2.3 Sample ethanol concentrations greater than the highest calibration level where the results are necessary for interpretation in the case shall be reanalyzed with a smaller sample amount (including dilution factor). Acceptable dilution factors for this method are 1:2 and 1:5. Alcoholic beverage proof dilutions have been assessed and fit for use at 1:50 and 1:100. The other targeted volatiles concentrations greater than the highest calibrator shall be reported as greater than 0.50 gm%.

8.1.8.2.4 The method’s calibration range is from the lower limit of quantitation (LLOQ) 0.01 gm% to the upper limit of quantitation (ULOQ) 0.50 gm% for ethanol, methanol, isopropanol and acetone. A weighted linear (1/x) model shall be used for all compounds.

> Note: Ethanol concentration of 0.80 gm% produced no carryover in validation studies. Volatile compounds concentration of 0.50 gm% for methanol, isopropanol, and acetone produced no carryover. Carryover should be individually evaluated in any case sample following a case where the volatile concentration exceeds the ULOQ for that batch. The scientist shall repeat the analysis if carryover above the ULOQ is suspected.

8.1.8.3 Volatile Results

8.1.8.3.1 Any quantitative or retention time result not used in a case shall either be lined through and initialed or all the data used shall be highlighted.

8.1.8.3.2 Qualitative results shall be expressed as “positive” or “negative” and include any clarifying remarks, if applicable.

8.1.8.3.3 For quantitative results, the average of the two test results shall be reported based on the following criteria:

- If the average of the two results is 0.05 gm% or above, a range shall be calculated by determining ±5% of the mean of the two test results.
- If the average of the two results is less than 0.05 gm%, a range shall be calculated by determining ±10% of the mean of the two test results.

8.1.8.3.4 When the two test results do not agree within the above stated percentages, the sample shall be retested in duplicate.

8.1.8.3.5 When the results do not agree after retesting, the examiner and/or Toxicology Unit Supervisor/Technical Leader should closely examine the case analysis to determine if possible problems such as sample degradation, internal standard variances, instrument variances, etc. could have affected the results. After careful review, a determination should be made as to whether any quantitative results can be reported or if the case must be reported qualitatively with an appropriate explanation.

8.1.8.3.6 All quantitative results shall be truncated to the thousandths digit.

8.1.8.3.7 All volatile results (excluding alcoholic beverages) shall be reported in weight/volume (gram%).

8.1.8.3.8 When a definitive conclusion cannot be made, the reason shall be documented on the report (e.g., “insufficient sample for analysis”, “sample unsuitable for analysis”, “results are inconclusive due to sample condition”, etc.).

8.1.8.3.9 Method validation determined that low sample volumes (<1mL) collected into grey stoppered tubes may cause the tested blood alcohol level to be lower than the actual result. Quantitative results may be reported in these cases with the appropriate clarifying remark.

8.1.8.4 Alcoholic Beverage Results

8.1.8.4.1 Alcoholic beverage results will be truncated after the tenths digit.

8.1.8.4.2 Alcoholic beverage results may be calculated using the following example, which is based on a one to one hundred dilution of the sample @ 22°C (density=0.78775).

1. Instrument results x 100 = gram %
2. Gram % x 1/density = v/v%
3. By combining 1 and 2 above, instrument results x (100 x (1/0.78775) = v/v%

8.1.8.4.3 Alcohol beverage results less than 1.0 v/v% will be considered not detected unless the circumstances of the case specifically require reporting these results.

8.1.9 References

Forensic Toxicology Laboratory Guidelines, Society of Forensic Toxicologists, American Academy of Forensic Sciences Toxicology Section, 1991.

Garriott, James C. *Medicolegal Aspects of Alcohol*, Lawyers & Judges Publishing Co., Tucson, AZ, 1996.

Kalin, Jack R. and Ezell, Anna L., *Forensic Ethanol Analysis and Interpretation*, Forensic Toxicologist Certification Board, Inc., 1997.

Weast, Robert C., *CRC Handbook of Chemistry and Physics,* Forty-Ninth Edition; The Chemical Rubber Co, Cleveland, Ohio, 1968; p F-3.

National Center for Biotechnology Information. PubChem Compound Summary for CID 3776, Isopropyl Alcohol. https://pubchem.ncbi.nlm.nih.gov/compound/Isopropyl-Alcohol. Accessed Apr. 13, 2023.