8.1 ALCOHOL PROCEDURE

8.1.1 Purpose

To qualitatively and/or quantitatively identify ethanol and other volatile substances 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
Burette
Thermometer
Test tube rotator
20 mm headspace vials
Crimp caps with septa
20 mm crimper
Hamilton diluter/dispenser
HS-GC/FID, HS-GC/MS, ChemStation software, compatible computer and printer

8.1.4 Reagents and Standards

Ethanol reference standard (NIST traceable)
Ethanol (CH_3CH_2OH)
Acetone ($(CH_3)_2CO$)
Isopropanol ($(CH_3)_2CHOH$)
Methanol (CH_3OH)
1,1-Difluoroethane ($C_2H_4F_2$)
Other reference standards (as needed)
0.01667 v/v% n-Propanol (internal standard)
Water (H_2O)

8.1.5 Standard Preparation

The following are examples of how to prepare the standards 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.

Ethanol Stock Reference Standard Solution [1 gm%] (A & B)
Add 12.69 mL of 99.5+% ethanol and dilute to 1000 mL with H_2O (@ 22°C).

MIAE Stock Reference Standard Solution [1v/v%/1 gm%] (A & B)
Add 12.69 mL of 99.5+% ethanol, 10 mL methanol, 10 mL isopropanol, and 10 mL acetone, and dilute to 1000 mL with H_2O (@ 22°C).
Note: 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 book.

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	AMOUNT USED	CONCENTRATION	TOTAL VOLUME
0.01	1 mL	MIAE Stock Soln.	100 mL
0.02	2 mL	MIAE Stock Soln.	100 mL
0.05	5 mL	MIAE Stock Soln.	100 mL
0.08	8 mL	Ethanol Stock Soln.	100 mL
0.10	10 mL	MIAE Stock Soln.	100 mL
0.20*	20 mL	MIAE Stock Soln.	100 mL
0.30	30 mL	MIAE Stock Soln.	100 mL
0.40	40 mL	Ethanol Stock Soln.	100 mL
0.50	50 mL	Ethanol Stock Soln.	100 mL

*If using this standard as a control, bubble 1,1 – difluoroethane through the solution.

8.1.6 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.

8.1.7 Reporting

Results can be reported if the following criteria are met:

8.1.7.1 Qualitative

8.1.7.1.1 Retention times of drugs 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.

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

8.1.7.2 Quantitative

8.1.7.2.1 All the qualitative result criteria above must be met.

8.1.7.2.2 Sample ethanol concentrations below 0.01 gm% (the lowest calibration point) shall be reported as "negative".

8.1.7.2.3 Other volatiles shall be reported when determined to be present at or above 0.01 v/v%.

8.1.7.2.4 Sample volatile concentrations greater than the highest calibration point shall be handled using the following methods:

- Reanalyze using smaller sample amounts (including dilutions).
- Reanalyze using higher standard concentrations.

8.1.7.3 Volatile Results

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

8.1.7.3.2 Qualitative results shall be expressed as "positive" or "negative" and include any clarifying remarks, if applicable.

8.1.7.3.3 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%/v/v% 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%/v/v%, a range shall be calculated by determining ±10% of the mean of the two test results.

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

8.1.7.3.5 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 analytical results can be reported or if the case must be reported qualitatively with an appropriate explanation.

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

8.1.7.3.7 Ethanol results (excluding alcoholic beverages) shall be reported in weight/volume (gram%).

8.1.7.3.8 All other volatile compound results shall be reported in volume/volume% (v/v%).

8.1.7.3.9 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.).

8.1.7.4 Alcoholic Beverage Results

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

8.1.7.4.2 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.

8.1.7.4.3 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 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.