ANALYSIS OF ALCOHOL AND OTHER VOLATILES BY HEADSPACE GC/FID

I. PRINCIPLE

The concentration of ethanol and common volatile analytes can be determined by headspace sampling and dual column gas chromatograph/flame ionization detection (GC/FID). Volatile components may be identified qualitatively or quantitatively.

II. SCOPE

This procedure can be used for the qualitative or quantitative analysis of ethanol, methanol, acetone and isopropanol in toxicology specimens.

III. SAFETY/QUALITY ASSURANCE

This procedure must be conducted in accordance with the Safety Manual, the Chemical Hygiene Plan and the Quality Manual.

All biological samples are treated with universal precautions. Appropriate personal protective equipment should be worn at all times. Flammable liquids and vapors may cause eye, skin and respiratory tract irritation. Material Safety Data Sheets (MSDS) are available in the laboratory.

IV. REAGENTS AND STANDARDS

Organic solvents are HPLC grade or higher and inorganic reagents (e.g. salts) are ACS grade. Deionized water is prepared using a Millipore Direct Q UV3 water system (or equivalent).

CALIBRATORS AND CONTROLS

1. Aqueous ethanol and mixed volatile calibrators are routinely prepared at the following concentrations: 0.020 g/100 mL, 0.040 g/100 mL, 0.100 g/100 mL, 0.200 g/100 mL, and 0.400 g/100 mL. These standards are used to generate an in-house calibration curve. The value of 0.000 g/100 mL will be derived by extrapolation from zero by the ChemStation software. Additional calibrators may be included as directed by Management.

2. In-house negative (0 g/100 mL) and positive aqueous and or/whole blood controls are included in each run. At least one internal and/or external aqueous and/or whole blood control containing ethanol at 0.080 g/100 mL is used to verify the calibration curve.

3. Internal and external controls for ethanol and other volatiles are prepared or purchased, depending on manufacturer availability. At a minimum, an external (aqueous or whole blood) control must be included in each run.

4. At least one mixed volatile control will be analyzed with each batch analysis to demonstrate that ethanol and other volatiles are capable of being separated and identified in an unknown mixture.

B. n-Propanol internal standard – An aliquot of an aqueous solution of n-propanol is added to each standard, control and unknown, to function as an internal standard.

V. PREPARATION OF INTERNAL STANDARD, CALIBRATORS, AND CONTROLS,

The preparation and verification of each internal standard, calibrator, and control should be documented using LAB-59 Volatiles Analysis Reagent Preparation Worksheet in accordance with the Quality Manual.

INTERNAL STANDARD PREPARATION

1. Weigh out 1.0 g of n-propanol in a 100 mL volumetric flask Q.S. to volume with deionized water. This will give you a 1.0% I.S. working stock solution (1.0 g/100 mL). Store refrigerated (6 month expiration).

2. Add 10 mL (10 mL = 0.10 g) of the I.S. working stock to a 1000 mL volumetric flask. Q.S. to volume with deionized water giving a 0.10 g/1000 mL or 0.010 g/100 mL I.S. solution which is 0.010%. Store refrigerated (6 month expiration). Document the preparation in the reagent logbook.

NOTE: Whenever a new lot of internal standard is prepared, the old lot should be discarded.

ETHANOL WORKING STOCKS

1. Prepare separate working stocks for ethanol calibrators and controls. To prepare the working stocks, weigh out 10 g of absolute (200 proof) ethanol or 10.52 g of 95% ethanol in a 100 mL volumetric flask. Q.S. to volume with deionized water; this will give a 10% ethanol working stock solution (10 g/100 mL). Label each solution appropriately. Store refrigerated (6 month expiration).

ETHANOL CALIBRATORS

1. Prepare Ethanol Calibrators. Add the following volumes (a-e) of the 10% ethanol calibrator working stock five 500 mL volumetric flasks. Bring to volume with deionized water. Store refrigerated (6 month expiration).

   a. 1 mL = 0.020 g/100 mL = level one (1) calibrator

   b. 2 mL = 0.040 g/100 mL = level two (2) calibrator

   c. 5 mL = 0.100 g/100 mL = level three (3) calibrator

   d. 10 mL = 0.200 g/100 mL = level four (4) calibrator

   e. 20 mL = 0.400 g/100 mL = level five (5) calibrator

2. External standards purchased from an approved vendor may also be used to prepare calibrators at appropriate concentrations.

ETHANOL CONTROLS - 0.080 g/100 ml

1. Aqueous – 0.080 g/100 mL
   Add 4 mL of the 10% ethanol control working stock solution to a 500 mL volumetric flask and bring to volume with deionized water. Store refrigerated (6 month expiration).

   2. Whole Blood – 0.080 g/100 mL
      To a 100 mL volumetric flask filled about half full with bovine blood; add 0.8 mL of the 10% ethanol control working stock solution, slowly, and with stirring. Bring to volume with bovine blood. Store refrigerated (6 month expiration).

   3. External standards purchased from an approved vendor may also be used as controls.

MIXED VOLATILES WORKING STOCKS

1. Prepare separate working stocks for mixed volatiles calibrators and controls. To prepare the working stocks, weigh out 10 g of isopropanol, acetone, absolute (200 proof) ethanol, and acetone in a 100 mL volumetric flask. Q.S. to volume with deionized water; this will give a 10% mixed volatiles working stock solution (10 g/100 mL). Label each solution appropriately. Store refrigerated (6 month expiration).

MIXED VOLATILES CALIBRATORS

1. Prepare mixed volatile calibrators. Add the following volumes (a-e) of the 10% mixed volatiles calibrator working stock five 100 mL volumetric flasks. Bring to volume with deionized water. Store refrigerated (6 month expiration).

   a. 0.2 mL = 0.020 g/100 mL = level one (1) calibrator

   b. 0.4 mL = 0.040 g/100 mL = level two (2) calibrator

   c. 1 mL = 0.100 g/100 mL = level three (3) calibrator

   d. 2 mL = 0.200 g/100 mL = level four (4) calibrator

   e. 4 mL = 0.400 g/100 mL = level five (5) calibrator

2. External standards purchased from an approved vendor may also be used to prepare calibrators at appropriate concentrations.

MIXED VOLATILE CONTROL

1. Prepare the mixed volatile standard by adding 1 mL of the mixed volatile control working stock to a 100 mL volumetric flask and Q.S. to volume with deionized water giving 0.100 g/100 mL (0.100%) solution of each volatile. Store refrigerated (6 month expiration).

2. External standards purchased from an approved vendor may also be used as controls.

VI. EQUIPMENT, MATERIALS AND REAGENTS

A. Gas Chromatograph with Flame Ionization Detector (Agilent 7890A) and supporting software, data collection and reporting system (Agilent ChemStation), and two different chromatographic columns suitable for alcohol analysis (e.g. Restek BAC-1 and Restek BAC-2, or comparable)

B. Headspace Autosampler (Agilent G1888)

C. Compressed gas cylinders (Helium, Hydrogen, Air and Nitrogen)

D. Vials, caps, stoppers, and crimpers

E. Positive displacement and/or air displacement micropipette and tips (20-200 µL, 100-1000 µL and 1-5 mL)

F. Volumetric flasks (50 mL, 100 mL, 500 mL and 1000 mL)

G. Analytical balance

H. Deionized water

I. Granular sodium chloride (NaCl)

J. Organic solvents: Ethanol, Methanol, Acetone, Isopropanol and n-Propanol (HPLC grade or higher)

K. Ethanol standard solutions (purchased or prepared)

L. n-Propanol internal standard solution

M. Volatile mixture standard

N. External aqueous and/or whole blood controls

VII. MAINTENANCE AND CALIBRATION OF LABORATORY INSTRUMENTATION

GENERAL REQUIREMENTS FOR ANALYTICAL INSTRUMENTATION

All instruments will be maintained in accordance with the manufacturer's recommendations and specifications. All instruments will be checked after being moved or if a major repair is performed. For ethanol only, analyze all

samples, calibrators and controls using method Ethanol.M. For common volatile analysis, analyze all samples, calibrators and controls using method Mixed.M.

GAS CHROMATOGRAPHY

Operating Conditions (as specified by methods Ethanol.M and Mixed.M).

Instrument acquisition using both these methods are identical. The methods differ only in the data analysis (report) generated. Ethanol.M generates a report for ethanol only; Mixed.M generates a report for ethanol, methanol, acetone and isopropanol.

1. Headspace Autosampler (Agilent G1888):

   Communications Mode: COM1
   Headspace vial Size: 10mL
   Carrier Gas: Helium
   Handshake Mode: Headspace Proceed
   Oven Stabilization Time: 1 min
   Oven Temperature: 70°C
   Loop Temperature: 85°C
   Transfer Line Temperature: 100°C
   Shake: Low
   Vial Equilibration Time: 12.0 min
   Vial Pressurization Time: 0.40 min
   Extractions per Vial: 1
   GC Cycle Time: 3.3 mins
   Inject Time: 1.00 mins
   Injection Volume: 1.0 mL
   Loop Equilibration Time: 0.10 min
   Loop Fill Time: 0.20 min

2. GC/FID (Agilent 7890A):

   Carrier Gas: Helium
   Oven Program: 40 °C for 2.9 minutes (isothermal)
   Mode: Split
   Split Ratio: 3.5:1
   Split Flow: 22.813 mL/min
   Inlet Temperature: 250°C

Inlet Pressure: 43.875 psi
Inlet Total Flow: 43.362 psi
Septum Purge Flow: 3 mL/min
Gas Saver: On (15 mL/min after 0.5 min)
Column Pressure: 43.875 psi
Column Flow: 17.549 mL/min
Holdup Time: 0.35714 min
Detector Temperature: 250°C
Hydrogen Flow: 30 ml/min
Air Flow: 400 mL/min
Makeup (Nitrogen) Flow: 25 mL/min

VIII. PROCEDURE

EVIDENCE EXAMINATION

A. If there is a valid evidentiary breath test, no further ethanol analysis is necessary at this time unless a specific request for analysis is made.

B. Retrieve blood alcohol evidence containers.

C. Document any discrepancies or irregularities.

D. Mark the innermost specimen container(s) with the laboratory case number and analyst's initials.

E. Record characteristics of the specimen (clotted, decomposed, etc.).

F. Following sample preparation and analysis, specimen container(s) should be repackaged in the original container as soon as practical. The evidence should be re-sealed in a manner that would detect tampering.

G. The evidence should be filed in an evidence storage area until its final disposition.

SAMPLE PREPARATION

1. Allow specimens, controls, and reagents to equilibrate to room temperature.

2. Prepare analysis vials for calibrators, unknown samples, controls, aqueous blanks (negative), and a mixed volatile standard by labeling with sample identifiers.

    a. One aliquot of each calibrator is prepared.

    b. Duplicate (or greater number of samples) are prepared for analysis (unless there is insufficient sample available for duplicate analysis).

    c. At least one 0.080 g/100 mL control sample is included immediately before and after the batch (sequence) run.

    d. At least one negative sample is run before and after the batch (sequence) run.

    e. At least one mixed volatile standard is included in each batch run.

3. Place ~1 g of sodium chloride in a 10 mL headspace vial.

4. Pipette 0.10 mL of specimen and 1 mL of internal standard solution to the vial. After the addition of the last component the cap should be secured and the vial swirled to ensure proper mixing of the contents.

5. Place the vial in the headspace auto-sampler carousel and document the position in the sequence log.

6. Include whole blood and/or aqueous controls at the beginning and end of each sequence as a quality control check.

7. A new pipette tip is used for each sample.

8. If clotted blood is analyzed, the entire sample may be re-homogenized and analyzed. Alternatively, the fluid in the specimen vial excluding the clot may be analyzed. It shall be expressly documented in the case report if analysis was performed on blood serum only.

9. Samples that contain ethanol at a concentration in excess of the highest calibrator may be reanalyzed using a smaller sample volume, dilution or higher calibrator consistent with acceptance criteria below. This must be clearly documented in the case folder. Alternatively, the sample may be

reported as "ethanol detected at greater than 0.400 g/100 mL."

10. If other tissues (liver, kidney, brain) are analyzed, fluid from the container is analyzed. If no fluid is available, mix 1 mL of water per gram of tissue sampled and homogenize. Analyze the resulting mixture, correcting the results for dilution, as needed. Analysis of this sample must follow analysis of the water used to certify that no ethanol is present in the water.

GAS CHROMATOGRAPHY ANALYSIS

1. Complete the sequence table for all samples and controls to be analyzed.

2. Analyze all calibrators, controls and specimens by GC utilizing the appropriate method (Ethanol.M or Mixed.M).

3. A new calibration curve is generated at the beginning of each days set of samples in the first (batch) sequence of that days set of requests.

4. Perform gas chromatography analysis with two different chromatographic columns.

5. Chromatograms of samples will be printed and retained in the case folder.

    a. Ethanol and n-propanol peak identification, retention time, peak area, and quantitation results must be included with the chromatograms. Chromatograms of standards and controls will be kept in a retrievable form in the laboratory.

6. Retain the instrumental parameters in the case file and/or in a retrievable form in the laboratory.

7. One or more controls (positive and negative) will be run at the beginning and end of the sequence. This will be recorded in the QC data on the worksheet LAB-51, Ethanol Confirmation Worksheet.

Expected Retention Times for Volatiles Ethanol.M and/or Mixed.M		
Volatile	**FID1/BAC2 RT***	**FID2/BAC1 RT***
Acetone	1.538	1.289
Ethanol	1.439	0.907

Isopropanol	1.681	1.078
Methanol	1.105	0.750

Retention time of air on the columns is as follows.

RTX-BAC2 on FID1 A is 0.677 min
RTX-BAC1 on FID2 B is 0.603 min

* Retention Times (RT) varies with column length

IX. ACCEPTANCE CRITERIA

Calibrators and Controls

1. In-house calibrators and controls must be within 10% of the target concentration or within the acceptance range defined by the manufacturer. For concentrations below 0.10 g/100 mL results must meet the above criteria or be within ± 0.005 g/100mL of the expected value. Controls must be within acceptable limits in order for casework to be reported.

2. The calibration curve must yield an R^2 value of 0.99 or greater.

Case Samples

1. Volatile substances detected in a sample on both columns may be reported qualitatively if retention times are within 2% of the verified standard on both columns.

2. Results from at least two aliquots should be available for evaluation unless precluded by limited sample availability, etc.

3. The reported concentration for a specific aliquot will be calculated as the average of the concentrations reported by each detector and truncated to no less than three decimal places.

4. Concentrations should be within 10% of each other or ± 0.02 g/100 mL for POSTMORTEM SAMPLES or within 5% of each other or ± 0.02 g/100 mL for ANTEMORTEM SAMPLES.

i. To calculate the variation of the samples use the equation:

$$\frac{\text{High value-Low value}}{\text{Low value}} \times 100$$

5. For concentrations less than 0.10 g/100 mL, results must meet the above criteria or be within ± 0.005 g/100mL of each other.

6. For samples that meet the above specifications, the reported value will be calculated as the combined average of the replicate specimens truncated to no less than three decimal places.

1. Conditions for reanalysis

   a. If the results do not meet the above specifications, the sample preparation and analysis should be repeated. If reanalysis is precluded by limited sample availability, the lowest value may be reported with approval of the Laboratory Manager.

   b. If the results exceed the highest calibrator, the analysis may be repeated with a diluted sample or a higher calibrator (if appropriate) provided that linearity is confirmed. If a dilution is performed, the requisite dilution correction is performed after the evaluation of the raw data.
   c. Linearity has been confirmed and verified 0.02 gm/100 mL to 0.80 gm/100 mL for ethanol.

X. REPORTING OF RESULTS

A. The results are reported in the following manner.

1. For volatiles in liquid samples, results are typically reported in grams of alcohol per 100 milliliters (g/100mL).

2. For volatiles in solid samples (i.e. tissue) the results should be reported in grams of alcohol per 100 grams of tissue (g/100g).

B. Levels below LOQ will be reported as negative or ethanol detected below LOQ (0.02 grams per 100 mL of blood) or equivalent statement.

D. If there is insufficient sample, the report should indicate "Insufficient sample for analysis" or equivalent statement.

E. If the sample is unsuitable for analysis, the report should indicate "Sample unsuitable for analysis" or equivalent statement.

F. If no volatile(s) are detected, the report should indicate "None detected" or an equivalent statement.

XI. CASE DOCUMENTATION

For preparation of the case folder, refer to Toxicology Case Records, TX-01-02 in the Standard Operating Procedure Manual.

XII. LITERATURE / SUPPOPTING DOCUMENTATION

1. Siek, T. J. 1972. Duplicate analysis of blood ethanol by injection onto two parallel gas chromatographic columns in rapid succession. *Journal of Forensic Sciences*, Number 2, April 1972.

2. Christmore, D. S., R. C. Kelly, and L. A. Doshier. 1984. Improved recovery and stability of ethanol in automated headspace analysis. *Journal of Forensic Sciences*, 29:1038-1044.

3. Method files: ETHANOL.M, MIXED.M

4. Lab Form LAB-51, Volatiles Confirmation Worksheet.

5. Lab Form LAB-59, Volatiles Reagent Preparation Worksheet.

6. Lab Form LAB-63, Volatiles Batch QC Data.

XIII. REVISION HISTORY

Revision #	Effective Date	Description of Change
01	08/07/2010	Original Issue
02	02/11/2011	Included criteria for postmortem samples and clarified acceptance criteria for all samples.
03	06/13/2011	Updated to include analysis of methanol, acetone and isopropanol.
04	09-10-2012	Corrected typo on injection volume on page 6. 1.0 min corrected to read 1.0 mL
05	11-20-2012	Updated header to Houston Police Department, Toxicology Section. Added option in reporting criteria to allow reporting of ethanol detected below LOQ (0.02 grams 100mL) in addition to negative in section X part B. Also simplified the naming of forms to Lab Form Number LAB-XX rather than prefaced with Sam Houston State University etc.

XIV. APPROVAL AND ISSUANCE

Assistant Laboratory Director

Irma Rios _____ Date: 11-20-2012

Criminalist Manager

Michael R Manes, MS _____ Date: 11-20-2012

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