Standard Operating Procedures

Analysis of Alcohol and Other Volatiles by Headspace GC/FID

Houston Forensic Science Center

Forensic Analysis Division

Toxicology Section – Alcohol Analysis

Version 0.1

Analysis of Alcohol and Other Volatiles by Headspace GC/FID

a) Principle

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

b) Scope

This procedure can be used for the qualitative or quantitative analysis of ethanol, methanol, acetone, and isopropanol in toxicology specimens. Routinely, specimens with an ethanol value of less than 0.100 g/100 mL are analyzed for drug toxicology.

c) Safety

This procedure must be conducted in accordance with the HFSC Health and Safety Manual and Quality Manual. All case specimens should be treated with Universal Bloodborne Pathogen Precautions. Appropriate personal protective equipment should be worn during sample and reagent preparation and when handling volatile or caustic chemicals. Material Safety Data Sheets (MSDS) are available in the laboratory.

d) Documentation

In addition to the requirements stated in the Quality Assurance Manual, any inconsistencies of a substantial nature noted in the case documentation, submission forms or evidence items will be documented and clearly articulated in the final report. This includes information such as the date of birth, location or date of offense, names and incident numbers. If there is any indication that an item could be associated with incorrect submission documentation, no work will be performed until the inconsistency has been clarified. If the inconsistency cannot be clarified in a timely manner, a report will be issued stating that no analysis has been performed and the reason for not analyzing the evidence. The HFSC reserves the right to track, audit, and report both major and minor inconsistencies in case documentation, submission forms, and other evidence items for quality assurance and monitoring purposes.

e) Calibrators and Controls

Appropriate controls, calibrators, and other standards may be purchased from an outside vendor for use in lieu of in-house prepared controls, calibrators, or other standards.

i) Aqueous ethanol and mixed volatile calibrators are routinely prepared or purchased at the following or similar concentrations: 0.020, 0.040, 0.100, 0.200, 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 the instrument software.

ii) Negative (0.000 g/100 mL) aqueous, positive aqueous, and whole blood controls are included in each run. At least one internal aqueous, external aqueous, or whole

blood control containing ethanol at 0.080 g/100 mL is used to verify the calibration curve.

iii) Ethanol and mixed volatile controls may be prepared or purchased, depending on manufacturer availability. At a minimum, an external control, either aqueous or whole blood, must be included in each run.

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

v) An aliquot of an aqueous solution of n-propanol is added to each standard, control, and case specimen, to function as an internal standard.

f) Preparation of Internal Standard (I.S.), Calibrators, and Controls

The preparation and verification of each internal standard, calibrator, and control is documented using LAB-59 (Volatiles Analysis Reagent Preparation Worksheet) or an equivalent method of documentation.

i) Internal Standard (I.S.) Preparation

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

(2) Add 10 mL (10 mL = 0.10 g) of the 1% I.S. stock solution to a 1000 mL volumetric flask. Bring to volume with deionized water giving a 0.100 g/1000 mL or 0.010 g/100 mL I.S. working solution which is 0.010%. Store refrigerated (6 month expiration).

ii) Ethanol Stock Solution

Prepare separate stock solutions for ethanol calibrators and controls. To prepare the stock solution, weigh out 10 g of absolute (200 proof) ethanol or 10.52 g of 95% ethanol in a 100 mL volumetric flask. Bring to volume with deionized water. This will give a 10% ethanol stock solution (10 g/100 mL). Solutions are labeled according to the requirements stated in the HFSC Quality Manual. Store refrigerated (6 month expiration).

iii) Ethanol Calibrators

To prepare ethanol calibrators, add the following volumes (1-5) of the 10% ethanol calibrator stock solution to five 100 mL volumetric flasks. Bring to volume with deionized water. Store refrigerated (6 month expiration). Other levels may be utilized with the approval of management or section supervisor. These levels may be included within the given range or to extend the curve.

(1) 0.2 mL = 0.020 g/100 mL = level one (1) calibrator
(2) 0.4 mL = 0.040 g/100 mL = level two (2) calibrator
(3) 1 mL = 0.100 g/100 mL = level three (3) calibrator
(4) 2 mL = 0.200 g/100 mL = level four (4) calibrator
(5) 4 mL = 0.400 g/100 mL = level five (5) calibrator

iv) Ethanol Controls

(1) Aqueous - 0.080 g/100 mL
Add 4 mL of the 10% ethanol control 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
Add 0.8 mL of the 10% ethanol control stock solution slowly, while stirring, to a 100 mL volumetric flask filled about half full with bovine blood, or equivalent. Bring to volume with bovine blood or equivalent. Store refrigerated (6 month expiration).

(3) Whole Blood (Alternative) – 0.080 g/100 mL
Add 0.8 ml of the 10% ethanol control stock solution to approximately 90 g of blood while stirring. Bring mass of control blood to 100 g by slowly adding blood.

(4) External standards purchased from an approved vendor may also be used as controls.

v) Mixed Volatile Stock Solution
Prepare separate stock solutions for mixed volatile calibrators and controls. To prepare the stock solution, weigh out 10 g of isopropanol, acetone, absolute (200 proof) ethanol, and methanol in a 100 mL volumetric flask. Bring to volume with deionized water; this will give a 10% mixed volatile stock solution (10 g/100 mL). Label each solution appropriately. Store refrigerated (6 month expiration).

vi) Mixed Volatile Calibrators
To prepare mixed volatile calibrators, add the following volumes (1-5) of the 10% mixed volatile calibrator stock solution to five 100 mL volumetric flasks. Bring to volume with deionized water. Store refrigerated (6 month expiration). Other levels may be utilized with the approval of management or section supervisor. These levels may be included within the given range or to extend the curve. Label each solution appropriately.

(1) 0.2 mL = 0.020 g/100 mL = level one (1) calibrator
(2) 0.4 mL = 0.040 g/100 mL = level two (2) calibrator
(3) 1 mL = 0.100 g/100 mL = level three (3) calibrator
(4) 2 mL = 0.200 g/100 mL = level four (4) calibrator
(5) 4 mL = 0.400 g/100 mL = level five (5) calibrator

vii) Mixed Volatile Control

(1) Aqueous – 0.100 g/100ml
Add 1 mL of the 10% mixed volatile control stock solution to a 100 mL volumetric flask and bring to volume with deionized water. Store refrigerated (6 month expiration).

g) Equipment, Materials, and Reagents

i) Instrumentation using a method that has been approved and validated for use in the section

ii) Compressed gas cylinders or equivalent (Helium, Hydrogen, Air, and Nitrogen)
iii) Vials, caps, stoppers, and crimpers
iv) Positive displacement and/or air displacement micropipette and tips (20-200 µL, 100-1000 µL, and 1-5 mL) or equivalent
v) Volumetric flasks (50 mL, 100 mL, 500 mL, and 1000 mL)
vi) Analytical balance
vii) Deionized water
viii) Granular sodium chloride (NaCl, ACS Grade)
ix) Organic solvents: Ethanol, Methanol, Acetone, Isopropanol, and n-Propanol (HPLC grade or higher)
x) Ethanol solutions
xi) n-Propanol internal standard solution
xii) Volatile mixture standard
xiii) External aqueous and/or whole blood controls
xiv) Homogenizer
xv) Centrifuge

h) Procedure
i) Evidence Examination
(1) If there is an indication that a breath test was conducted, no further ethanol analysis is necessary at this time unless a specific request explaining the need for the additional analysis is made.
(2) Retrieve blood alcohol evidence containers.
(3) Document any substantial discrepancies or irregularities.
(4) Mark the innermost specimen container(s) with the laboratory case number, sub-item number, the analyst's initials, and the date.
(5) The condition of the specimen analyzed will be recorded (e.g., normal, thick, clotted, decomposed).
(6) Following sample preparation and analysis, specimen container(s) should be repackaged in the original container as soon as practical.
(7) The evidence shall be stored in an appropriate evidence storage area until its final disposition.
ii) Sample Preparation
(1) Allow case specimens, controls, calibrators, and reagents to equilibrate to room temperature.
(2) Prepare headspace vials for calibrators, case specimens, and controls by labeling with sample identifiers.
(a) One aliquot of each calibrator is prepared.
(b) A minimum of two aliquots of each case specimen are prepared for analysis, unless there is insufficient sample available for duplicate analysis.
(c) At least one 0.080 g/100 mL control is included before and after the batch (sequence) run.

(d) At least one negative control is included before and after the batch (sequence) run.
(e) At least one mixed volatile control is included in each batch (sequence) run.
(f) At least one whole blood control is included at the beginning and end of each batch (sequence) run.

(3) Place ~1 g of sodium chloride in each vial.
(4) Pipette 100 µL of specimen and 1 mL of internal standard solution into the vial. After the addition of the last component, the cap should be secured by crimping and the vial swirled to ensure proper mixing of the contents. A new pipette tip is used for each specimen where appropriate. Ensure that the cap is tightly affixed to the vial and there are no cracks in the vial.
(5) The vial must be placed in the headspace autosampler carousel in correlation with the instrument sequence table.
(6) If there is an indication of a clot, the specimen should be addressed by one of the following:
(a) Homogenize the specimen (e.g., sonication, pipette shearing).
(b) The specimen may be spun down and analyzed as serum/plasma.
(7) 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 specimen must follow analysis of the water used, to certify that no ethanol is present in the water.
(8) When alcoholic beverages or other liquids are analyzed, fluid from the container is diluted appropriately based on suspected alcohol content prior to analysis.
(a) Example: For a 4% alcoholic beverage, a 1:20 dilution is appropriate.
(b) Commonly used dilutions are as follows:

Dilution	Beverage (µL)	Deionized Water (µL)
1:20	50	950
1:50	20	980
1:100	10	990

(c) From the dilution, a 100 µL aliquot is analyzed.

iii) Gas Chromatography Analysis
(1) Complete the sequence table for all specimens and controls to be analyzed.
(2) Analyze all calibrators, controls, and specimens by GC utilizing the appropriate method.
(3) A new calibration curve is generated at the beginning of each day's set of samples in the first sequence/batch of that day.
(4) Chromatograms of the case specimens will be retained in the case record.

(5) Ethanol and n-propanol peak identification, retention time, and quantitation results must be included with the chromatograms. Chromatograms of calibrators and controls will be kept in a retrievable format in the laboratory.
(6) The controls (positive and negative) will be recorded. This may be done using the Lab 51 (Ethanol Confirmation Worksheet) or an equivalent record.
(7) Specific instrument parameters and conditions are documented for each specific instrument used. Verification data will be kept in a retrievable format in the laboratory.

i) Acceptance Criteria
  i) Calibrators and Controls
    (1) Calibrators and controls must be within 10% of the target concentration or 0.010 g/100ml, whichever is greater, or within the acceptance range defined by manufacturer.
    (2) Individual analyte values in each control must be within acceptable limits in order for the analyte to be reported.
    (3) If a control is not within the given range, all specimens bracketed by that control must be reanalyzed.
    (4) If any control is not within 5% or 0.005 g/100 mL, whichever is greater, a pipette verification must be conducted to ensure the pipette is functioning as expected. This will be documented and kept in a retrievable format in the laboratory.
    (5) The calibration curve must yield an R^2 value of 0.99 or greater.
  ii) Case Specimens
    (1) Volatile substances detected in an aliquot on both columns may be reported qualitatively if retention times are within 2% of the verified standard on both columns.
    (2) To determine the ethanol concentration of a case specimen, all four analytically obtained values from the two aliquots (alq) will be combined to provide the mean ethanol concentration using the following formula:

calculated mean = [(alq1A + alq1B + alq2A + alq2B)/4]

All four values must be within 5% or 0.005 g/100 mL of the calculated mean, whichever is greater. The following formula may be used to calculate the percentage.

$$percentage = \left(\frac{abs(calculated\ mean - value)}{calculated\ mean}\right) * 100$$

    (3) Post-mortem specimens should be within 10% of each other or ± 0.02 g/100 mL.

(4) For serum/plasma specimens, the calculated mean will be divided by a conversion factor of 1.2 to calculate the equivalent whole blood concentration that will be reported.
(5) For beverages or other liquids, the calculated mean will be multiplied by the dilution factor and reported to three significant figures.
(6) The final reported value for all case specimens, except beverages or other liquids, will be truncated to three decimal places.

iii) Conditions for reanalysis
(1) If the above specifications are not met, the sample preparation and analysis should be repeated. If reanalysis is precluded by limited specimen availability, the lowest value may be reported or the specimen may be reported as "Quantity not sufficient" with approval of the section supervisor.
(2) If the results exceed the highest calibrator, the analysis may be repeated with a diluted specimen 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. Alternatively, with the approval of management or section supervisor, the sample may be reported as "Ethanol detected at greater than 0.400 g/100 mL" or an equivalent statement.

j) Reporting of Results
i) The results are reported in a following manner.
(1) For volatiles in liquid specimens, results are typically reported in grams of alcohol per 100 milliliters of liquid (g/100 mL).
(2) For volatiles in solid specimens (i.e. tissues), the results should be reported in grams of alcohol per 100 grams of tissue (g/100 g).
(3) For serum/plasma specimens, the report should state "This sample was centrifuged prior to analysis. The reported value was calculated from the determined serum/plasma value of ____ to its whole blood equivalent using a conversion factor of 1.2" or equivalent statement.
(4) For specimens that required homogenization, the report should state "This sample was homogenized prior to analysis" or an equivalent statement.
ii) Levels below the lowest calibrator (0.020 g/100 mL) will be reported as "none detected," "less than 0.020 g/100 mL," or an equivalent statement
iii) If there is insufficient sample, the report should state "Insufficient sample for analysis" or equivalent statement.
iv) If the sample is unsuitable for analysis, the report should state "Sample unsuitable for analysis" or equivalent statement.
v) If no volatile(s) are detected, the report should state "none detected" or an equivalent statement.