1. Principle

This procedure is used to analyze biological specimens for volatile compounds (e.g., methanol, ethanol, acetone, and isopropanol), utilizing a headspace-gas chromatographic (HS-GC) technique followed by detection using flame ionization (FID). Samples are typically in an aqueous environment, are sealed within air-tight vials, and are examined using a headspace analysis-type sampling technique. Volatile components within aqueous solutions, when placed in closed heated environments, reach an equilibrium between their vapors and their solutions. Vapor phases are analyzed with appropriate columns and detectors (e.g., HS-GC/dual FID, dual column technique). Volatile components of solutions are identified and can be subsequently quantitated using calibration graphs, reference standards, and internal standards. Most samples being analyzed by this method are related to driving under the influence (DUI) investigations or Drug Facilitated Crimes (DFC).

2. Specimens

This procedure mainly uses biological specimens (e.g., blood, serum/plasma and urine), however liquids may also be analyzed. When available, 0.1 mL of sample is used within this procedure (2 aliquots of specimen, 50 µL each).

Specimens with elevated analyte concentrations may require dilution with water to ensure that results are within the linear range of the calibration graph used in the procedure (i.e., 0.01 g% to 0.40 g%).

All liquid samples should be prepared using a 1:100 dilution prior to sampling of 50 uL.

If sample amount is limited then modifications to this procedure are allowed (e.g., analyzing only one aliquot, dilution) with approval of a FSE2 or higher and will be documented within applicable case file(s).

3. Reagents

3.1 If a part number or item number listed is not available, an equivalent may be purchased.

3.2 All reagents must be ACS grade or better.

3.2.1	Water	Millipore, Deionized (DIW)
3.2.2	n-Propanol	ThermoFisher Scientific 043848
3.2.3	Multicomponent Alcohol Calibration Kit (C1-C6)	Cerilliant A-127
3.2.4	80 mg/dL Aqueous Ethanol Standard Solution	Lipomed LPM-ETH-080-1ML
3.2.5	Alcohol Mixture 4	Lipomed LPM-MIX-1808-1LH
3.2.6	0.08 % Ethanol-Water Solution	NIST SRM 2893a

4. Equipment

4.1 General laboratory glassware

4.2 Headspace autosampler vials (20 mL) with appropriate magnetic caps, septa and crimper

4.3 Hamilton pipettor-diluter

4.4 Vortexer

4.5 Headspace-gas chromatograph with flame ionization detectors (HS-GC dual (FID)) (Agilent 7890B or equivalent)

4.6 Capillary Column to the back detector (Rtx-BAC1; Restek 18003, or DB-BAC1 UI; Agilent 123-9334UI, 30m, 0.32mm id, 1.8 µm, or equivalent)

4.7 Capillary Column to the front detector (Rtx-BAC2; Restek 18002, or DB-BAC2 UI; Agilent 123-9434 UI, 30m, 0.32mm id, 1.2 µm, or equivalent)

5. Preparation of Solutions

Externally prepared calibrator and control solutions will be used; a typical batch will contain a negative control, a Cerilliant calibration curve, a 0.08 g% Lipomed control and a 0.10 g% Lipomed control. The NIST 0.08 g% control will be run quarterly and with proficiency tests.

Note: Controls from a different manufacturer (e.g. Cerilliant) can be used, if necessary, but a FSE2 (or higher) approval is required prior to batch analysis.

New lots of reagents, calibrator solutions, control solutions, and other types of solutions will be analyzed and validated prior to being used on casework samples for reporting purposes. These new lots of solutions will typically be analyzed along with older (previously validated) solutions. See TX 43 Tox Quality Control Manual for guidance.

Externally Prepared Solutions:

5.1 Open and individually transfer solution into autosampler vial

5.1.1 Store tightly capped and refrigerated; stable for one (1) week after opening.

5.1.2 Manufacturer expiration dates are only valid for unopened solutions.

n-Propanol Internal Standard Solution:

5.2 Transfer 280 µL of n-propanol into a 2.0 L volumetric flask

5.3 Dilute to volume with DIW and mix well

5.4 Transfer to an amber storage container and properly label

5.4.1 Store tightly capped at room temperature; stable for one (1) year.

6. Procedure

6.1 Let all specimens and solutions come to room temperature

6.2 Label vials for calibrators and controls as seen below (variations are acceptable upon approval of a FSE2 (or higher)):

6.2.1 Blank DIW (no internal standard)

6.2.2 Negative Control (contains DIW and Internal Standard)

6.2.3 0.01 g% Volatile Calibrator

6.2.4 0.025 g% Volatile Calibrator

6.2.5 0.05 g% Volatile Calibrator

6.2.6 0.20 g% Volatile Calibrator

6.2.7 0.40 g% Volatile Calibrator

6.2.8 Blank DIW (no internal standard)

6.2.9 0.08 g% EtOH Positive Control

6.2.10 0.10 g% Volatile Positive Control

6.2.11 Evidentiary Samples

6.2.11.1 Each evidentiary sample will be analyzed in duplicate

6.2.12 0.08 g% EtOH Positive Control: will be analyzed after each full set of twelve (12) evidentiary samples or less (each replicate is considered an evidentiary sample).

6.2.13 0.10 g% Volatile Positive Control

6.2.14 Blank DIW (no internal standard)

Pipettor/Diluter Preparation/Priming:

6.3 Turn on the Pipettor/Diluter and check for bacterial contamination in solutions

6.4 Place the inlet tubing into the NPA Internal Standard Solution bottle making sure the end of the tubing is well below the level of the liquid.

6.5 Remove the dispenser probe from its holder and place the tubing from the probe in an empty waste container/flask.

6.6 Press the prime switch and liquid will be dispensed from the probe at this time. The system's lines will be primed. Continue to cycle until the bubbles have disappeared from the lines.

Sample preparation with Pipettor/Diluter:

6.7 Use the automated pipettor and place its probe into blank DIW, draw up 50 µL, dispense into a waste container, and perform the between-sampling rinse. Dry the probe tip with a clean wipe as needed.

6.8 Press the Pipette Activation Button (PAB) once to draw up 450 µL of the NPA Internal Standard Solution (This may occur automatically depending on automated pipettor programming).

6.9 Place the probe into the sample to be analyzed and press the PAB again to draw up 50 µL.

6.10 Place the probe inside the appropriately labeled vial and press the PAB once to dispense.

6.11 Place the probe into a waste container and press the PAB once more to dispense the between-sampling rinse into the waste container. Dry the probe tip with a clean wipe as needed.

6.12 Place a headspace cap on the vial and crimp-seal tightly. Take this time to verify that the proper sample was transferred into the correspondingly labeled headspace vial.

Repeat the internal standard/sample dispensing into head-space vials for all calibrators, controls, and samples. This can be done according to the order stated earlier under this 'procedure' section.

Note: If the pipettor is not functioning then manual pipetting is permitted with the approval of a FSE2 or higher. Adequate adjustments will be performed by analysts so that this procedure can be followed.

Setting-up Instrument with Samples:

6.13 Ensure air and hydrogen are flowing and ensure detectors (flame ionization detectors (FID) are operational).

6.14 A negative control and 0.10 g% volatile calibrator or control are analyzed prior to casework. Both samples should demonstrate appropriate peaks and responses. The instrument must have passing QA/QC results prior to preparing and loading samples.

6.15 Enter the samples and vial positions in the sequence

6.16 Enter the following dilution factors:

6.16.1 Urine: 0.769

6.16.2 Serum/Plasma: 0.862

6.16.3 Blood: 1.000

6.17 Place the labeled headspace vials in the appropriate order within the instrument

6.18 Save the sequence to the day's date.

6.19 Prior to analyzing samples:

6.19.1 Print the sequence list.

6.19.2 Check that the physical placement of the headspace vials and the vial positions within the instrument's sequence list match.

6.19.3 Once the check has been completed place an indication of the sample check (e.g., 'sequence checked' or 'sequence verified') on the sequence page along with analyst's initials and date.

6.20 Print the instrument method and include both the method and the sequence printouts with the batch documents.

6.21 Begin the sequence and analyze the samples.

7. Instrumental Parameters

The following parameters can be used. Some values may change due to slight variability (e.g., flow rates). Significant differences must be approved by a FSE2 (or above) prior to changing.

Autosampler	Parameters		Autosampler	Parameters
GC Cycle Time	6.5 min.		Sample Vial Penetration	15 mm
Sample Volume	0.5 mL		Sample Vial Penet. Speed	50 mm/sec
Incubation Time	5 min.		Sample Aspiration Rate	12 mL/min
Incubation Time Increment	0 min.		Sample Post Asp. Delay	1 sec.
Heat Agitator	On		Inlet Penetration Depth	45 mm
Incubation Temperature	50 C		Inlet Penet. Speed	50 mm/sec
Heat Syringe	On		Pre-Inject Time Delay	0.5 sec.
Pre-Injection Flush Time	5 sec.		Inject Flow Rate	10 mL/min.
Agitator Speed	250 rpm		Post Injection Delay	0.5 sec
Agitator On Time	5 sec.		Flush Time	10 sec.
Agitator Off Time	5 sec.		Continuous Flush	Off
GC	**Parameters**		**Front Inlet**	**Parameters**
Run Time	6.5 min.		Mode	SPLIT
Post Run Time	0 min.		Split Ratio	10:1
Oven Equilibration Time	0 min.		Split Flow	36.575 mL/min.
Max. Oven Temperature	250 C		Heater	250 C
Initial Temperature	44 C		Pressure	5.8565 psi
Hold Time	6.5 min.		Total Flow	43.232 mL/min.
Post Run Temperature	100 C		Septum Purge Flow	3 mL/min.
Carrier Gas	H_2		Gas Saver	On

Column Information		
Column Names	Col. #2 (BAC1)	Col. #3 (BAC2)
Film Thickness	1.8 µm	1.2 µm
Column Length	30 m	30 m
Inner Diameter	320 µm	320 µm
Column Max Temp	260°C	260°C
Pressure (initial)	5.2 psi	5.2 psi
Pressure (post run)	3.8 psi	3.8 psi
Flow	2.1193 mL/min.	2.1516 mL/min.
Ave. Velocity	40.3 cm/sec.	40.606 cm/sec.
Holdup Time	1.2407 min.	1.2314 min.
Detector	**FID2 (Back Detector)**	**FID1 (Front Detector)**
Temperature	250 C	250 C
Sampling Rate	20 Hz (Signal #1)	20 Hz (Signal #1)
Makeup Gas	N2	N2
Makeup Flow	25 mL/min.	25 mL/min.
H_2 Flow	25 mL/min.	25 mL/min.
Air Flow	400 mL/min.	400 mL/min.

8. Decision Criteria

The following criteria are used in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), peak shape, and signal-to-noise (aka: integrable peaks) are used as the basis for peak detection. In most cases, the criteria below will be met to detect the appropriate volatile analytes within specimens.

8.1 Chromatography

Chromatographic peaks will possess good chromatographic quality (i.e., Gaussian peak shape, reasonable peak width, distinguishing signal-to-noise). For a chromatographic peak to be deemed acceptable it will compare favorably to corresponding chromatographic peaks within known samples (i.e., positive controls). The retention time of a chromatographic peak in a case sample should be within ±0.1 minute of the retention time obtained from analysis of a calibrator or positive control.

8.2 Batch Acceptance

8.2.1 The batch must have completed with no unexplained interruptions and no unexplained errors. Consult a FSE2 (or higher) if unsure whether to continue processing the batch.

8.2.2 All calibrator solutions that have been analyzed during a batch (and their resulting data) will be included in data processing and sample evaluation. No calibrators within a batch can be selectively eliminated from the resulting calibration graph without AD approval and will be documented appropriately within the batch.

8.2.3 All applicable analytes of interest within positive controls, as well as internal standards, will be identified during data processing.

8.2.3.1 Analytes of interest are compounds that are being reported.

8.2.4 To report results on a specimen, the internal standard response must be within +/- 10% of a calibrator or control.

8.2.5 Quantitation of data may be performed automatically by the software. While quantitation is normally performed using BAC1 column data, processing using BAC2 data can be performed at the discretion of a FSE2 (or higher).

8.2.6 Quantitative calculations are based on comparisons of chromatographic peak areas between analytes and the internal standard.

8.2.7 Peak area response ratios from the calibrator solutions are calculated and compiled to create a linear calibration curve (y-axis is Area Ratio and x-axis is ethanol concentration). A best-fit line ($y = mx+b$) is created from the calibration graph which does not include the origin as a point.

8.2.8 Calibrator and control solution data should be evaluated prior to analyzing evidentiary samples to ensure that quality control measures have been satisfied and the calibration graph is acceptable.

8.2.9 Calibration curves are considered acceptable if:

8.2.9.1 $R^2 \geq 0.99$

8.2.9.2 Quantitative results are within +/- 20% of the target value

8.2.9.3 If a calibration curve is not acceptable then the batch is rejected for that analyte and case samples will not be quantitatively reported within that batch.

8.2.10 Positive control results are considered acceptable if:

8.2.10.1 Quantitative results for EtOH are within +/- 5% of the target value:

8.2.10.1.1 0.08 g% range: 0.076 g% - 0.084 g%

8.2.10.1.2 0.10 g% range: 0.095 g% - 0.105 g%

8.2.10.2 Quantitative results for MeOH, Acetone, and IPA are within +/- 10% of the target value:

8.2.10.2.1 0.10 g% range: 0.090 g% - 0.110 g%

8.2.10.3 Quantitative results of the 0.08 g% NIST EtOH positive control are within the uncertainty range for EtOH.

8.2.10.4 If positive control values are found to be outside their designated acceptable limits, a FSE2 (or higher) will be consulted.

8.2.10.5 Positive control results are recorded in the appropriate Quality Control (QC) chart spreadsheet.

8.2.11 Other than the n-propyl alcohol (NPA internal standard), no significant peaks will be present in the negative control sample. Significant peaks are considered peaks which are automatically integrated, and which impact the quality of the results.

8.2.12 Column (BAC1) Sample Replicate Evaluation (for EtOH only):

8.2.12.1 The difference between the BAC1 column results for each aliquot replicate should be within +/- 5% of the mean value (using 3 decimal places). If the above criteria are not met, seek the appropriate FSE2 (or higher) guidance to determine if samples need to be re-analyzed or if acceptance of current value is allowed – document in batch case notes.

8.2.13 Dual Column Evaluation:

8.2.13.1 Any reportable analyte must be able to be identified by retention times on both GC columns.

8.2.13.2 All reportable GC peaks should be totally resolved.

8.2.13.3 To be reportable, analytes must have quantitative values from both GC columns (BAC1 and BAC2) that agree within +/- 10 % of each other.

8.2.14 Reporting Limit (RL):

8.2.14.1 The reporting limit for volatiles will be 0.02 g%. Anything below 0.020 g% but greater than or equal to 0.010g% will be reported as "Detected less than 0.02 g%"

8.2.14.2 Blood samples with ethanol concentrations greater than the highest calibrator solution (e.g., > 0.40 g%) will be reanalyzed using appropriate dilutions if quantitation is needed. Information regarding how the dilutions were prepared will be noted within the batch paperwork. If unsure of the proper dilution consult a FSE2 (or higher).

8.2.14.3 Urine samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.30 g% will be reanalyzed using appropriate dilutions if quantitation is needed. Information regarding how the dilutions were prepared will be noted within the batch paperwork. If unsure of the proper dilution consult a FSE2 (or higher).

8.2.14.4 Serum/plasma samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.34 g% will be reanalyzed using appropriate dilutions if quantitation is needed. Information regarding how the dilutions were prepared will be noted within the batch paperwork. If unsure of the proper dilution consult a FSE2 (or higher).

8.2.14.5 Based on toxicological significance, samples with other volatile analytes that are found to be outside the calibration ranges will be assessed to determine if they need to be diluted and repeated or if they can simply be reported either qualitatively or greater than the highest calibrator. A FSE2 (or higher) will be consulted for such instances.

8.2.14.6 Other cases where samples cannot be repeated (e.g., limited sample) will be noted within batch and casefile paperwork. A FSE2 (or higher) will be consulted for such instances.

8.2.14.7 Samples which contain significant carry-over peaks (i.e., contamination into the next sample) may need their samples re-analyzed. A FSE2 (or higher) will be consulted for such instances.

8.2.14.8 Difluoroethane (DFE) and other commonly abused inhalants may be detected by this method. They may be reported qualitatively if a peak is observed in both sample aliquots on both columns. The relative retention times shall be within 0.02 min of a reference standard.

9. Reporting of Results

The following criteria are used as guidelines in determining how to report results using this procedure.

9.1 For evidentiary biological samples where volatile analytes were quantitated and were within the reportable limits (RL):

9.1.1 The reported value will be the lower of the two (2) BAC1 column replicate concentrations.

9.1.2 Whole blood concentration numbers will be truncated to two (2) decimal digits.

9.1.3 Urine sample ethanol concentration values will be converted to whole blood equivalent ethanol values (i.e., BAC equivalent) using a conversion ratio of 1.3–to–1 (i.e., 0.769).

9.1.4 Serum/Plasma sample ethanol concentration values will be converted to whole blood equivalent ethanol values (i.e., BAC equivalent) using a conversion ratio of 1.16–to–1 (i.e., 0.862).

9.2 Ethanol concentration values within non-biological samples will not have any conversions performed and will be reported qualitatively (i.e Detected)

9.3 The uncertainty value must have the same number of decimal digits as the measured quantity, therefore measurement uncertainty will be calculated using the three (3) decimal place concentration and listed on the final report in addition to the truncated concentration.

10. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment will be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use. Refer to Safety Manual for further guidance.

11. References

Reed, D. and Cravey, R.H. (1971) A Quantitative Gas Chromatographic Method for Alcohol Determination. J. Forensic Sci. Soc. 11:263

Karnitis, L. and Porter L.J. (1971) A Gas Chromatographic Method for Ethanol in Vapors of Biological Fluids. J. Forensic Sci. 16:318-322

Jones, A.W. and Schubereth, J. (1989) Computer-aided Headspace Gas Chromatography Applied to Blood Alcohol Analysis: Importance of Online Process Control. J. Forensic Sci. 34:1116-1127

Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man*, 7th ed., Biomedical Publications: Foster City, California, 2004.

Moffat, A.C., *Isolation and Identification of Drugs*, 2nd ed., Pharmaceutical Press: London, 1986.

Dubowski, K.M., *Manual for Analysis of Ethanol in Biological Liquids*, 1977.

Garriott, James, *Medicolegal Aspects of Alcohol*, 3rd ed., Lawyers and Judges Publishing: Tucson, AZ, 1996.

Levine, B.; Smith, M.L.; Smialek, J.E.; Caplan, Y.H. *J For Sci*. 1993, *38*, 663-667.