Title: Analysis of Biological Specimens for Volatile Compounds using Headspace-Gas Chromatograph/Mass Spectrometry (Flame Ionization Detection) {HS-GC/MS (FID)}

1. Introduction

This procedure is primarily used to detect, identify, and quantitate ethanol and other volatile compounds within biological matrices (e.g., blood, urine, vitreous humor). The majority of samples being analyzed by this method are related to driving under the influence (DUI) investigations. Presumptive screening for ethanol within such samples can be accomplished using an Enzyme-Multiplied Immunoassay Technique (EMIT) instrument. Even though the analysis for ethanol using the EMIT instrument is a non-immunoassay experiment, any samples that are screened positive by this instrumentation will be analyzed using this procedure for confirmation. Additionally, samples suspected of containing volatile components other than ethanol (e.g., methanol, acetone, isopropanol) can also be analyzed using this procedure.

2. Scope

This procedure allows for the screening, quantitation, and confirmation of ethanol, methanol, acetone, and isopropanol within biological samples. Other biological matrices can be utilized using this procedure once the procedure has been properly validated. The procedure does not necessarily need to be updated when used with new matrices. Additional matrices can be used and chemicals other than the four previously listed can be sought, if needed, using this procedure when proper quality assurance/quality control (QA/QC) measures are utilized and decision criteria are met.

3. Principle

This procedure involves the analysis of samples for volatile compounds, (e.g., methanol, ethanol, acetone, isopropanol, and similar compounds), utilizing a headspace-gas chromatographic (HS-GC) or headspace-gas chromatographic/mass spectrometric (HS-GC/MS) method. 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/FID, HS-GC/MS, dual column technique). Volatile components of solutions are identified and can be subsequently quantitated using calibration graphs, reference standards, and internal standards.

The headspace technique is based on Henry's law which state that when a volatile liquid in solution (e.g., ethanol) comes into contact with a closed air space, an equilibrium forms between the liquid phase and the headspace of that solution. At a constant temperature the concentration of the volatile liquid within the headspace is directly proportional to its concentration within the liquid phase. This procedure describes a way to separate, identify, and quantitate, if necessary, analytes (i.e., volatiles) from biological matrices.

4. Specimens

Any aqueous sample may be suitable for this analysis including, but not limited to: blood, serum, urine, vitreous humor. Blood sample collection tubes containing proper anticoagulant and preservative (i.e., 'gray-tops' containing potassium oxalate and sodium fluoride) should be used. However, any airtight tubes will be accepted. Containers for urine and other sample types should be within sealed, preferably airtight, glass or plastic (e.g., polypropylene) containers. All samples should be received with proper documentation, have been properly sealed (i.e., prevent sample loss, contamination, or deleterious change), and have been properly stored. Once samples are received within the Toxicology Unit all corrections regarding proper packaging or evidence integrity will be corrected prior to being properly stored within either a refrigerator or freezer.

When available, 0.4 mL of specimen is typically used within this procedure (2 aliquots of specimen, 200 µL each). Specimens with elevated analyte concentrations (e.g., high ethanol concentrations based on EMIT data) may require dilution with water in order to ensure that results are within the linear range of the calibration graph used in the procedure (i.e., 0.01 g% to 0.4 g%).

If sample amount is limited then modifications to this procedure are allowed (e.g., analyzing only one aliquot, dilution). Additionally, specimens other than biological materials may be analyzed using this procedure. However, if either of these situations occur then either the Unit Lead or higher needs to document approval within the applicable case file(s) prior to analysis.

5. Equipment/Materials/Reagents

5.1 General laboratory glassware (e.g., Class A glassware)

5.2 Headspace autosample vials (e.g., 20 mL) with appropriate seals/caps and crimper.

5.3 Pipettes (e.g., automatic pipettor-diluter), or equivalent

5.4 Vortex mixer

5.5 Headspace-gas chromatograph with flame ionization detectors (HS-GC(FID)), Shimadzu (or equivalent) or Headspace-gas chromatograph with mass spectral detector (HS-GC/MS)), Agilent (or equivalent)

5.6 Capillary Column #1: (Rtx-BAC1 ; Restek 18003, 30m, 0.32mm i.d., 1.8 µm, or equivalent)

5.7 Capillary Column #2 (Rtx-BAC2 ; Restek 18002, 30m, 0.32mm i.d., 1.2 µm, or equivalent)

5.8 Acetone (HPLC grade, or equivalent)

5.9 Deionized water (DIW, Millipore, or equivalent)

5.10 Ethanol (EtOH ; anhydrous 200 proof USP, or equivalent)

5.11 $\text{Ethanol}_{(aq)}$ (80 $\text{mg/dL}_{(aq)}$; Certified Reference Material (CRM) Standard, Cerilliant E-030, or equivalent)

5.12 Multicomponent Mixture (MeOH, EtOH, Acetone, IPA ; 0.1 g$\%_{(aq)}$; 100 mg/d$L_{(aq)}$; 1 mg/m$L_{(aq)}$; Certified Reference Material (CRM), Cerilliant A-056, or equivalent)

5.13 Isopropanol (IPA ; HPLC grade, or equivalent)

5.14 Methanol (MeOH ; HPLC grade, or equivalent)

5.15 n-Propanol (NPA ; HPLC grade, or equivalent)

5.16 Sodium Azide (NaN_3 ; Baker, or equivalent)

5.17 Volatile Mixture (MeOH, EtOH, Acetone, IPA ; in water
0.01 g$\%_{(aq)}$, 0.025 g$\%_{(aq)}$, 0.05 g$\%_{(aq)}$, 0.2 g$\%_{(aq)}$, 0.4 g$\%_{(aq)(aq)}$;
Certified Reference Material (CRM), Cerilliant (A-127), or equivalent))

6. Preparation of Calibrators, Controls and Standard Stock Solutions

The externally-prepared ethanol positive control CRM at 0.080 g% $(w/v)_{(aq)}$ will be analyzed within batches – regardless if internal or external solutions are utilized. If this CRM is unavailable then the Lead Examiner (or higher) will be notified and, upon written approval, an equivalent positive control can be used. This will be documented in the notes within each batch.

Externally-prepared calibrator and control solutions will be primarily used. Comparable in-house solutions will be used when external solutions are not available.
For example, a typical batch will include the following (not in any particular order):

a) Externally-Prepared [5-Point] Volatile [CRM] Calibrator Solutions:
(0.010 g%, 0.025 g%, 0.050 g%, 0.20 g%, 0.40 g%)

b) Externally-Prepared Ethanol [CRM] Positive Control Solution (0.08 g%)

c) Externally-Prepared Volatile [CRM] Positive Control Solution (0.10 g%)

d) Negative Control Solution (Externally prepared or In-House)

e) Unknown Samples

New lots/batches of reagents, calibrator solutions, control solutions, and other types of solutions will be analyzed and validated for quality (and documented) prior to being used on casework samples for reporting purposes. These new lots/batches of solutions/material will typically be analyzed along with older (previously validated) reagents/solutions so that they can be quality-compared and validated as being acceptable for use.

Note: Additional positive controls (other than what are listed) can be used, whether they be externally purchased or internally prepared. Different external calibrator solutions can be used, if necessary, but both the appropriate FSE2 and AD (or higher) approval is required prior to batch analysis.

Note: Lot numbers on labels for solutions/reagents internally generated will be of the format: month, day, year, analyst's initials, and a sequential number: {Example: 08112018MPR}

If multiple lots are made on the same date by the same analyst then a sequential number will be added: {Example: 08112018MPR-01, 08112018MPR-02}

Note: Throughout the procedure different volumes/amounts can be used as long as the same final concentration is obtained. If different volumes are used, however, such differences will be noted on the batch summary sheet under the ‘Notes’ section. The appropriate FSE2 (or higher) should be notified just to make sure nothing in procedure will be negatively impacted.

6.1 Check balance using calibrated weights on the day of solution preparation. Record appropriate checks accordingly.

Externally-Prepared Volatile Calibrator Solutions:

6.2 Externally-Prepared [5-Point] Volatile [CRM] Calibrator Solutions
(Volatile Mixture: $MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$)
(0.010 g% (w/v), 0.025 g% (w/v), 0.050 g% (w/v), 0.20 g% (w/v), 0.40 g% (w/v))

6.2.1 Carefully open (e.g., break top of glass ampule), individually transfer liquid into autosample vial, cap, and securely seal.
The solutions should be: 100 µg/mL, 250 µg/mL, 500 µg/mL, 2000 µg/mL, and 4000 µg/mL mixtures of MeOH, EtOH, Acetone, and Isopropanol in 1.2 mL ampules. No dilutions are necessary.

6.2.2 Cap and securely seal after each use. Store refrigerated.

6.2.3 Label appropriately and store refrigerated. Manufacturer expiration dates are only valid for unopened solutions. When refrigerated, opened solutions are stable for one (1) month from the date of their last validation (i.e., comparison to controls from a separate lot/batch).

Externally-Prepared Positive Control Solutions:

6.3 $Ethanol_{(aq)}$ [CRM] Positive Control Solution ($EtOH_{(aq)}$; 0.080 g% $(w/v)_{(aq)}$)

6.3.1 Carefully open (e.g., break top of glass ampule), individually transfer liquid into autosample vial, cap, and securely seal.
The solution should be: 80 µg/mL of EtOH in 1.2 mL ampules. No dilutions are necessary.

6.3.2 Cap and securely seal after each use. Store refrigerated.

6.3.3 Label appropriately and store refrigerated. Manufacturer expiration dates are only valid for unopened solutions. When refrigerated, opened solutions are stable for one (1) month from the date of their last validation (i.e., comparison to calibrators from a separate lot/batch).

6.4 Externally-Prepared Volatile [CRM] Positive Control Solution
($MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$; 0.10 g% $(w/v)_{(aq)}$)

6.4.1 Carefully open the ampule and transfer the liquid into an autosample vial.

6.4.2 Cap and seal after use.

6.4.3 Label the vial appropriately.

6.4.4 Store refrigerated.

Internal Standard (I.S.) Solutions:

6.5 n-Propanol [$NPA_{(aq)}$] Internal Standard (I.S.) Stock Solution (5.6 g% $(w/v)_{(aq)}$; 7.0 % $(v/v)_{(aq)}$)

6.5.1 Transfer 7.0 mL of n-propanol into a 100 mL volumetric flask, dilute to volume with DIW, and mix well. (Note: density of NPA is 0.803 g/mL)

6.5.2 Transfer to an amber bottle (or equivalent) and properly label.

6.5.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) year from the date of its last validation.

6.6 n-Propanol [$NPA_{(aq)}$] Internal Standard Working Solution (0.011g% $(w/v)_{(aq)}$; 0.014 % $(v/v)_{(aq)}$)

6.6.1 Transfer 4.0 mL of the $NPA_{(aq)}$ I.S. Stock Solution into a 2.0 L volumetric flask, dilute to volume with DIW, and mix well.

6.6.2 Transfer to an amber bottle (or equivalent) and properly label.

6.6.3 This solution will be tightly capped and can be stored at room temperature. It is stable for one (1) year from the date of its last validation.

In-House (Internal) Volatile Calibrator Solutions (used when External Solutions are not available):

6.7 In-House [3-Point] Volatile Calibrator Solutions
($MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$)
(0.02 g% (w/v), 0.10 g% (w/v), 0.30 g% (w/v))

6.7.1 Volatile Calibrator Stock Solution (VCSS ; 1.0 g% $(w/v)_{(aq)}$ each of MeOH, EtOH, Acetone, IPA)

6.7.1.1 Accurately weigh 5.000g (+/- 0.002g) of acetone, methanol, isopropanol, and ethanol.
Quantitatively transfer into a 500 mL volumetric flask with DIW.
Add 0.1 g of sodium azide, dilute to volume with DIW, and mix well.

6.7.1.2 Transfer the VCSS to an amber bottle (or equivalent) and properly label with lot number and other information (a properly capped volumetric flask with parafilm is considered equivalent).

6.7.1.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) month from the date of its last validation.

6.7.2 Low Volatile Calibrator Solution (LVCS ; 0.02 g% $(w/v)_{(aq)}$; 0.020 g/100 mL)

6.7.2.1 Using a volumetric pipette add 2 mL of the 1.0 g% (w/v) Volatile Calibrator Stock Solution to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.7.2.2 Transfer to an amber bottle (or equivalent) and properly label.

6.7.2.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) month from the date of its last validation.

6.7.3 Medium Volatile Calibrator Solution (MVCS ; 0.1 g% $(w/v)_{(aq)}$; 0.10 g/100 mL)

6.7.3.1 Using a volumetric pipette add 10 mL of the 1.0 g% (w/v) Volatile Calibrator Stock Solution to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.7.3.2 Transfer to an amber bottle (or equivalent) and properly label.

6.7.3.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) month from the date of its last validation.

6.7.4 High Volatile Calibrator Solution (HVCS ; 0.3 g%$(w/v)_{(aq)}$; 0.30 g/100 mL)

6.7.4.1 Using volumetric pipettes add 30 mL of the 1.0 g% (w/v) Volatile Calibrator Stock Solution to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.7.4.2 Transfer to an amber bottle (or equivalent) and properly label.

6.7.4.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) month from the date of its last validation.

<u>In-House Positive Control Solutions</u>:

6.8 In-House Ethanol Positive Control Solution ($EtOH_{(aq)}$; 0.5 g% $(w/v)_{(aq)}$; 0.50 g/100mL)

6.8.1 Accurately weigh 5.000g (+/- 0.002g) of ethanol and quantitatively transfer into a 1000 mL volumetric flask with DIW containing 0.2 g of sodium azide.
Dilute to volume with DIW and mix well.

6.8.2 Transfer to an amber bottle (or equivalent) and properly label.

6.8.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) month from the date of its last validation.

6.9 In-House Volatile Mix Positive Control Solution (VPCS)
($MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$; 0.10 g% $(w/v)_{(aq)}$; 0.10 g/100 mL)

6.9.1 Prepare in same manner as the Volatile Calibrator Stock Solution (VCSS ; 1.0 g% $(w/v)_{(aq)}$ each of MeOH, EtOH, Acetone, IPA) except use reagents from a different source (or lot #) if available. Transfer the newly prepared solution to an amber bottle (or equivalent) and properly label as In-House Volatile Mix Positive Control Stock Solution (VPCSS). This solution will be tightly capped and stored refrigerated (a properly capped volumetric flask with parafilm is considered equivalent). It is stable for one (1) month from the date of its last validation.

6.9.2 Using a volumetric pipette add 10.0 mL of the VPCSS (1.0 g% $(w/v)_{(aq)}$) to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.9.3 Transfer the VPCS to an amber bottle (or equivalent) and properly label (a properly capped volumetric flask with parafilm is considered equivalent).

6.9.4 This solution will be tightly capped and stored refrigerated. It is stable for one (1) month from the date of its last validation.

<ins>Negative Control Solution</ins>:

6.10 Negative Control Solution (0.00 g% $(w/v)_{(aq)}$ of MeOH, EtOH, Acetone, IPA)

6.10.1 Place known DIW into a 1000 mL volumetric flask containing 0.2 g of sodium azide. Dilute to volume with DIW and mix well. Alternate variations of preparing this solution are acceptable (e.g., 250 mL of water with 50 mg sodium azide).

6.10.2 Transfer into an amber bottle (or equivalent) and properly label.

6.10.3 This solution will be tightly capped and stored refrigerated. It is stable for one (1) year from the date of its last validation.

7. Procedure

7.1 Place all specimens and solutions at a room temperature environment for approximately thirty (30) minutes.

7.2 Prepare or acquire necessary calibrator and control solutions.

7.3 Label vials for calibrators and controls as seen below (variations are acceptable upon approval of the appropriate Lead Examiner (or higher)):

7.3.1 Blank DIW (no internal standard)

7.3.2 Negative Control Solution (contains I.S.)

7.3.3 Volatile Calibrator (e.g., External [CRM] 0.010 g%)

7.3.4 Volatile Calibrator (e.g., External [CRM] 0.025 g%)

7.3.5 Volatile Calibrator (e.g., External [CRM] 0.050 g%)

7.3.6 Volatile Calibrator (e.g., External [CRM] 0.20 g%)

7.3.7 Volatile Calibrator (e.g., External [CRM] 0.40 g%)

7.3.8 Blank DIW (no internal standard)

7.3.9 Positive Control EtOH Solution (e.g., External Ethanol [CRM] 0.08 g%)

7.3.10 Volatile Positive Control (e.g., External 0.10 g%)

7.3.11 Blank DIW (no internal standard)

7.3.12 Evidentiary Samples ($X_i \ldots X_n$) ; will be made in duplicate.
Two (2) aliquots (200 µL) of each evidentiary item (i.e., blood, urine, vitreous) will be placed into separate headspace vials.

7.3.13 Volatile Positive Control (e.g., External 0.10 g%)
Will be analyzed after each full set of twelve (12) evidentiary items or less (each replicate specimen vial is considered an evidentiary item). The frequency of the number of samples analyzed before a Volatile Positive Control is analyzed can be altered with the appropriate Lead Examiner's (or higher) permission.

7.3.14 Volatile Positive Control (e.g., External [CRM] 0.10 g%) ; next-to-last sample in batch.

7.3.15 Blank DIW ; last sample in batch.

Note: Internally prepared calibrators are acceptable if external calibrators are unavailable.

Note: There is nothing precluding multiple sets of batches from different Examiners from being analyzed within the same instrumental sequence as long as each Examiner prepares their own calibrator and control samples.

Sample Preparation (Automatic Pipetting of Samples, Calibrators, and Controls):

Pipettor/Diluter Preparation/Priming:

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

7.5 Place the inlet tubing into the NPA Internal Standard working solution bottle making sure the end of the tubing is well below the level of the liquid.

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

7.7 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:

7.8 Use the automated pipettor and place its probe into blank DIW, draw up 200 µL, dispense into a waste container, and perform the between-sampling rinse. Dry the probe tip with a clean wipe as needed.

7.9 Press the Pipette Activation Button (PAB) once to draw up 1 mL of the $\text{NPA}_{(aq)}$ (Internal Standard Working Solution).

7.10 Place the probe into the sample to be analyzed (i.e., negative control, calibrator, positive control, unknown sample) and press the PAB again to draw up 200 µL.

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

7.12 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.

7.13 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.

7.14 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 automatic pipettor is not functioning then manual pipetting is permitted. Adequate adjustments will be performed by analysts so that this procedure can be followed. The appropriate Lead Examiner (or higher) can be sought for assistance, if necessary.

Setting-up Instrument with Samples:

7.15 Ensure air and hydrogen are flowing and ensure detectors (flame ionization detector (FID) and/or mass spectrometer (MS) are operational.

7.16 Ensure the appropriate instrumental quality assurance/quality control (QA/QC) procedures were performed. The instrument must have passing QA/QC results prior to preparing and loading of samples.

7.17 Enter the sequence order for the method Batch Table.

7.18 Place the labeled headspace vials in the appropriate order within the instrument.

7.19 Enter the following dilution factors:
0.769 for urine, 0.862 for serum, and 1.000 for blood and vitreous samples.

Note: OCME samples do not contain dilution factors.

7.20 Save the Batch Table to the day's date.

7.21 Prior to analyzing samples:
Print the sequence list. Check that the physical placement of the headspace vials and the vial positions within the instrument's sequence list match. 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 Analysts initials and date.

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

7.23 Begin the sequence and analyze the samples.

7.24 After the batch has completed:
Re-check that the physical placement of the headspace vials and the vial positions within the instrument's sequence list match. Once the re-check has been completed place an indication of the sample re-check (e.g., 'sequence re-checked post analysis' or 'sequence re-verified post analysis') on the sequence page along with Analysts initials and date.

8. Instrumentation

Instrumental Parameters:

The following are the typical operating parameters for the instrument used in this procedure. With approval from the Lead Examiner and Assistant Director (or higher), the instrument conditions may be modified to adjust or improve the procedure. Documentation of such changes must be included with batch data so that any instrumental parameter change can be associated with data and casework until this procedure has been updated.
The appendix contains an abbreviated version of the procedure. The checklist can be used by analysts. Any changes within the instrumental parameters, if listed on the checklist, will be reflected on the checklist by the analyst filling it out.

HS-GC(FID/MS):
The following parameters can be used. Some values may change due to slight variability (e.g., flow rates). Significant differences must be approved by the appropriate Lead Examiner (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**		**Injector**	**Parameters**
Run Time	6.5 min.		Mode	SPLIT
Post Run Time	0 min.		Split Ratio	10:1
Oven Equilibration Time	0 min.		Split Flow	46.049 mL/min.
Max. Oven Temperature	250 C		Injector Heater	250 C
Initial Temperature	47 C		Injector Pressure	9.5 psi
Hold Time	6.5 min.		Injector Total Flow	53.654 mL/min.
Post Run Temperature	100 C		Septum Purge Flow	3 mL/min.
			Gas Saver	On
Transfer Line	**Parameters**			
Temperature	200 C			

HS-GC(FID/MS) (Cont'd):

Column Information	**Agilent**	**Agilent**
Column Names	DB-BAC-1 Ultra Inert (Col. #2)	DB-BAC-2 Ultra Inert (Col. #3)
Column Cat. #	123-9334UI	123-9434UI
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)	7.9 psi	7.9 psi
Pressure (post run)	3.8589	3.8589 psi
Flow	2.6857 mL/min.	1.5733 mL/min.
Ave. Velocity	59.637 cm/sec.	27.599 cm/sec.
Holdup Time	0.8384 min.	1.8117 min.
Detector	**MS**	**FID (Front Detector)**
Temperature (Source)	230 C	250 C
Temperature (Quad)	150 C	–
Sampling Rate	–	20 Hz (Signal #1)
Makeup Gas	–	He
Makeup Flow	–	25 mL/min.
H_2 Flow	–	30 mL/min.
Air Flow	–	400 mL/min.
Ion Source	EI	–
Fixed Electron Energy	70 eV	–
Solvent Delay	1 min.	–
Gain Factor	2	–
EM Saver	False	–
Acquisition Type	Scan	–
Start Mass	29 m/z	–
End Mass	200 m/z	–
Threshold	0	–
Scan Speed	781 (N=3) Scan Speed	–

9. Decision Criteria

The following criteria are used as guidelines 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 all of the criteria below will be met in order to detect the appropriate volatile analytes within specimens. Only analytes found within evidentiary specimens of batches will need to be evaluated. For example, if twenty-five (25) unknown samples are analyzed and ethanol is the only volatile analyte detected at reportable limits, then the other volatiles which are in the controls and calibrators need not be processed nor evaluated.

9.1 Chromatography

Chromatographic peaks will possess good chromatographic quality (i.e., Gaussian peak shape, reasonable peak width, distinguishing signal-to-noise). In order for a chromatographic peak to be deemed acceptable it will compare favorably to corresponding chromatographic peaks within known samples (i.e., positive controls). The relative retention time of a chromatographic peak (i.e., compared to the internal standard's retention time) will be within ±0.1 minute of the relative retention time obtained from analysis of a reference standard or positive control.

9.2 Mass Spectrometry

In order for analytes to be reported, correct mass spectra for each analyte will be present. Ion ratios should compare favorably to ion ratios from applicable reference standards. Generally, ion ratios are within the limits as specified within the Section procedure related to mass spectral comparisons.

9.3 Batch Acceptance

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

9.3.2 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 within out AD approval and documentation explaining why and how such elimination is statistically allowable.

9.3.3 No analytes of interest will have been detected in the Negative Control.

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

Note: Analytes of interest are considered those compounds that are being reported.

9.3.5 Calibration Check (EtOH-only): Quantitative results of the External Ethanol [CRM] ; 0.080 g%) Positive Control Solution must be within +/- 5% of its target value (range: 0.076 g% – 0.084 g%). Results from the CRM data are recorded in the appropriate Quality Control (QC) chart spreadsheet.

9.3.6 Calibration Checks (MeOH, EtOH, Acetone, IPA): Quantitative EtOH results within the Volatile Positive Control Solution must be within +/- 5% of its target value (i.e., 0.10 g% ; range: 0.095 g% – 0.105 g%) and quantitative MeOH, acetone, and IPA results within the Volatile Positive Control Solution must be within +/- 10% of their target values (i.e., 0.10 g% ; range: 0.090 g% – 0.110 g%). Results from these data are recorded in the appropriate Quality Control (QC) chart spreadsheet.

9.3.7 Calibration Checks (other volatiles): Quantitative results from other positive controls (other than from ethanol and the volatile controls) which may have been analyzed along with associated calibration solutions shall be within +/- 10% of their targeted values. Results from these data are recorded in the appropriate Quality Control (QC) chart spreadsheet.

9.3.8 Other than the n-propyl alcohol (NPA internal standard), no significant peaks will be present in the negative control sample. A significant peak is an analyte of interest with an extrapolated concentration ≥ 0.005 g% .
{Blank and Carryover Check}

Note: Significant peaks are considered peaks which are automatically integrated and which impact the quality of the results.

9.3.9 Analytes below the lowest calibrator will not be quantitatively reported. However if such analytes are chromatographically/mass spectrometrically acceptable (and toxicologically significant) then they may be reported qualitatively.

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

9.3.10.1 Analyte's empirical concentration is ≤ 0.03 g%:
The difference between the BAC1 column results for each aliquot replicate should be ≤ 20 %. 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.

9.3.10.2 Analyte's empirical concentration is between 0.03 g% and 0.08 g%:
The difference between the BAC1 column results for each aliquot replicate should be ≤ 10 %. 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.

9.3.10.3 Analyte's empirical concentration is ≥ 0.08 g%:
The difference between the BAC1 column results for each aliquot replicate should be ≤ 5 %. 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.

9.3.10.4 If blood specimens are clotted and the above criteria are not met then the samples should be centrifuged and be analyzed as serum samples. Seek the appropriate FSE2 (or higher) guidance to determine if samples need alternative processing.

9.3.10.5 If the above criteria are not met and it is not due to clotting, then a Unit Lead or higher will be consulted and the samples may need to be repeated. Record within the case notes of the batch and, if necessary, within affected casefile notes.

9.3.11 Dual Column Evaluation:

9.3.11.1 Any reportable analyte must be able to be identified by relative retention times (i.e., peak times compared to the internal standard) on both GC columns.

9.3.11.2 All reportable analyte GC peaks should be totally resolved. Extracted ion chromatography (mass spectral data) will be used if co-elution is an issue.

9.3.11.3 In order to be reportable, analytes must have quantitative values from both GC columns (BAC1 and BAC2) that agree within +/- 10 %.

9.3.12 Unknown Sample Compound Detection:

This procedure is intended to be used for both qualitative and quantitative purposes of select analytes (e.g., ethanol). The majority of other unknown compounds will only be qualitatively reported. If necessary, other analytes may be quantitated (e.g., acetone).

9.3.13 Reporting Limit (RL):

9.3.13.1 Only analytes at or above the lowest calibrator solution will be reported quantitatively. Analytes that have been automatically integrated from the processing software but whose concentrations are below the lowest calibrator solution may be reported qualitatively. For quantitative purposes the reporting limit for volatiles will be 0.02 g%.

9.3.13.2 If analytes have quantitation values greater than the highest calibrator solution then dilution and re-analysis should occur. Variations in reporting terminology can also be used if appropriate and approved by the Unit Lead or higher (e.g., Item #01: Ethanol ≥ 0.40 g%).

9.3.13.3 Blood samples with ethanol concentrations greater than the highest calibrator solution (e.g., > 0.40 g%) will be reanalyzed using appropriate dilutions if accurate quantitations above that highest calibrator's concentration are needed. Information regarding how the dilutions were prepared will be noted within the batch paperwork. If unsure of the proper dilution consult the Unit Lead (or higher).

9.3.13.4 For urine samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.30 g%, appropriate dilutions and re-analyses are necessary if the highest calibrator solution is 0.40 g% (> 0.23 g% in In-House calibrants are used). Notes describing how the dilutions were prepared will be in the batch paperwork. If unsure of the proper dilution then consult the Unit Lead (or higher).

9.3.13.5 For serum samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.34 g%, appropriate dilutions and re-analyses are necessary if the highest calibrator solution is 0.40 g% (> 0.25 g% in In-House calibrants are used). Notes describing how the dilutions were prepared will be in the batch paperwork. If unsure of the proper dilution then consult the Unit Lead (or higher).

9.3.13.6 Based on toxicological significance, samples with 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. The Unit Lead (or higher) will be consulted for such instances and notes will be kept within batch documentation.

9.3.13.7 Other causes where samples are not able to be repeated (e.g., limited sample) will be noted within batch and casefile paperwork. The appropriate FSE2 and higher will be consulted on how to phrase report so that such instances are captured within the report as to why certain QA/QC actions were not performed.

9.3.13.8 Samples which have analytes that elute after a GC run has completed (e.g., past 4 minutes) may need to be reanalyzed using longer GC run times.

9.3.13.9 Samples which contain significant carry-over peaks (i.e., contamination into the next sample) may need their samples re-analyzed. Consult Unit Lead (or higher) when these situations occur. Documentation in case files will occur to explain rationale of decision of whether to re-analyze or not.

9.3.14 Data Processing:

9.3.14.1 Quantitation of the headspace-GC data may be performed automatically by the software. This is often done using macros. While quantitation is normally performed using BAC1 column data, if applicable and if necessary, GC data processing using BAC2 data can be performed. Acceptability of data using BAC2 data will be at the discretion of the appropriate FSE2 (or higher) and will be documented in case notes.

9.3.14.2 Quantitative calculations are based on comparisons of chromatographic peak areas between analytes and the internal standard (i.e., n-propanol).

9.3.14.3 Peak area response ratios from the calibrant solutions are calculated and compiled to create a linear calibration graph (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.

9.3.14.4 Calibrator solutions only need to be analyzed once per instrumental sequence/batch.

9.3.14.5 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.

9.3.15 Other Criteria:

Blank and Carryover Check: Other than the n-propyl alcohol (NPA internal standard), no significant peaks will be present in the negative control sample. If any reportable target analytes are present in the blanks or negative control samples then they should be at concentrations below 0.005g%.

Note: Toxicologically significant refers to whether the reporting of an analyte would reasonably have an impact to the customer.

9.3.16 Calibration Linearity: All calibration lines are considered acceptable if their associated coefficient of determination (R^2) are ≥ 0.99. The calibration lines for each analyte are independently evaluated as follows:

9.3.17 If a calibration line is not acceptable then the batch is rejected for that particular analyte and case samples will not be quantitatively reported until a new calibration line is acquired and deemed acceptable.

9.3.18 Troubleshooting, instrument evaluation, and/or preparation of new calibration solutions, if necessary, will be done prior to analysis of samples.

9.3.19 If some of the repeated ethanol positive control values are found to be outside their designated acceptable limits then casework samples that were analyzed prior-to and directly-after (i.e., those analyzed in-between bracketed controls) will be re-analyzed, if possible. Consult the appropriate Unit Lead (or higher) for guidance, if necessary.

9.3.20 Other than ethanol, if volatile positive control analyte's results are found to be outside designated acceptable limits then consult the appropriate Unit Lead (or higher) for guidance on how to proceed.

9.3.21 Appropriate corrective action(s) may need to be done before casework samples are reported if multiple control values are found to be unacceptable (e.g., repeat entire batch after performing instrument QA/QC). Consult the appropriate Unit Lead (or higher) for guidance, if necessary.

10. Reporting of Results

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

10.1 Analyte levels less than the reportable limit will be reported as '[Analyte(s)] not reportable' (or similar language). Further description of when such phrases are used will be included in reports so as to convey to readers that analytes may be present at concentrations lower than their reportable limits.

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

10.2.1 The reported value will be an average of the two (2) BAC1 column replicate concentrations.

10.2.2 Whole blood concentration numbers will be truncated to three (3) decimal digits (i.e., 3 decimal places).

10.2.3 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).

10.2.4 Serum 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).

Note: For OCME cases, ethanol concentrations within samples will not be converted.

10.3 Ethanol concentration values within non-biological samples will not have any conversions performed and will be reported by weight (i.e., g%). Reporting an ethanol concentration within non-biological samples will involve converting the weight concentration value to a volume concentration value using an ethanol density of 0.789 g/mL, thus getting a volume-volume percent concentration. (Example: (0.080 g% / 0.789 g/mL) = 0.10 % (v/v))

10.4 Uncertainty of measurement will be reported based on the most current calculated uncertainty value for this procedure.

10.5 The format for reporting volatile analyte concentrations will be: y +/- U (where y is the measured quantity value and U is the expanded uncertainty).

10.6 A confidence interval of at least 95% will be used.

10.7 Measurement uncertainty will be reported to three (3) decimal digits (i.e., 3 decimal places) because listing this number with only two (2) decimal digits would cause uncertainty values of zero (i.e., 0.00) to be reported. Such values would cause confusion for customers. Because the uncertainty value must have the same number of decimal digits as the measured quantity, three (3) decimal digits will be used for both the concentration value and the associated measurement uncertainty within this procedure.

Quality Assurance/Quality Control (QA/QC)

10.8 Each sample is evaluated for acceptability according to the sample and run acceptance criteria specified above.

10.9 Sensitivity: The limit of quantitation (LOQ) for this procedure has been administratively set to be 0.010 g% (w/v) for ethanol, methanol, isopropanol, and acetone. The reporting limit has been administratively set to be at 0.020 g% (w/v) for the same analytes for this procedure. Other volatile compounds will have LOD and LOQ values listed within their validation documents. The limit of detection (LOD) for ethanol, methanol, isopropanol, and acetone for this procedure has been administratively set to at least 0.010 g% (w/v).

10.10 Specificity: Because of the dual column concept, no potentially interfering substances have been identified using this procedure. All samples are analyzed using two (2) separate columns (using differing polarity chemistries) as well as two (2) different detectors (flame ionization and mass spectrometry).

10.11 Accuracy: The use and practice of analyzing positive controls contemporaneously with evidentiary samples ensures that correct volatile compounds are detected and reported.

10.12 Precision: Positive control concentrations are empirically calculated, stored within a spreadsheet (i.e., QC charts) and tracked for uniformity and repeatability/reproducibility.

10.13 Linearity: Best-fit calibration lines between the ranges of 0.010g% to 0.40g% for ethanol, methanol, isopropanol, and acetone are recorded and evaluated within each batch.

10.14 Sample Verification: All vial sequences and vial positions are checked both before and after samples are analyzed by the instrument. Evidence, when enough is available, is aliquoted and sampled at least two-times within each batch.

11. 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.

12. References

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

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

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Appendix:
Bench Method:
Volatile Analysis by HS-GC(FID/MS)

The following is an abbreviated version of this procedure and a pdf-version can be used.
This form may be used at the bench level by the analyst performing the procedure.

Negative Control [volatile-free]$_{(aq)}$	**Lot:**	
Positive Controls$_{(aq)}$ Externally Prepared / In-House	**Volatile Mix:** **Ethanol:**	**Lot:** **Lot:**
n-Propanol Internal Standard	**Lot:**	
Calibrator Solutions$_{(aq)}$ Externally Prepared / In-House (circle one)	**Calib. #1:** **Calib. #2:** **Calib. #3:** **Calib. #4:** **Calib. #5:**	**Lot:** **Lot:** **Lot:** **Lot:** **Lot:**
Other:	**Type:** **Type:** **Type:** **Type:** **Type:**	**Lot:** **Lot:** **Lot:** **Lot:** **Lot:**

Procedure:

For Blood or Urine (or Other Matrix) Specimens	
Ensure gas flows are adequate and instrument is warmed-up and ready for analyses (QA/QC is complete)	
Place samples, controls, and calibrators at room temperature for ~ 30 minutes	
Prepare or acquire calibrator/control solutions	
Label vials appropriately (calibrators, controls, evidence samples, blanks)	
Prepare automatic Pipettor/Dilutor and prime (if using)	
Prepare samples (i.e., 1 mL of I.S.(n-propanol$_{(aq)}$) + 200 µL sample)	
Between-sample rinse (if automatic Pipettor/Dilutor is used)	
Tightly cap headspace vials	
Repeat dispensing as necessary	
Create sequence within instrument software	
Ensure methods and parameters are correct	
Ensure appropriate dilution factors are set (OCME do not get dilution factors)	
Save the batch table to today's date	
Verify accuracy of sequence/vial placement (prior to analysis):	
Verify accuracy of sequence/vial placement (post analysis):	

Rev. #	History
3	Updated verbiage and formatting within the entire document. Appendices were updated with correct information and formatting was changed. Added ‘Blank DIW’ (that doesn’t contain an internal standard) within the Automatic Pipetting of Samples, Calibrators, and Controls step. Updated instrumental parameters including adding separate HS-GC-2 parameters. Combined the volatile stock solution to one mixture (Volatile Working Solution). Renamed old 'Blank‘ to be ‘Negative Control’ (contains internal standard). Replaced ‘Conditioner’ to ‘0.02 g% Volatile Working Solution’ within the instrumental sequence. Changed the calibration requirements to not include the origin (0-point). Added a GC ramp pre-conditioning step, if necessary, within the Instrumental Analysis section. Added HS-GC-2 information within the Instrumental Analysis section. Changed ‘Run Evaluation’ to Decision Criteria’ and updated that section. Requirements within ‘Sample Acceptance Criteria’ section was updated. Throughout document: Eliminated much redundancy, Director changed to Deputy Director, Supervisor changed to Lead Examiner. Previous cross-outs were removed and font color within entire document was changed to black.
4	6.1.3 Results for the EtOH CRM standard must be within 5% of its target 0.08% value, range 0.076 – 0.084%. 6.1.4.4 Accuracy and Precision: All positive control solution quantitation results must agree within 10% of the statistical mean of their respective concentration. Added 0.1g% Ethanol Control - directions to prepare. Added statement that controls and calibrators will be made from different stock solutions. Clarified statement on when dilutions are needed. Added statement that the analysts will use the EMIT results for urine samples as a guide to determine if a sample must be diluted.
5	Updated the SOP to allow for a combined volatile mixture for the calibration of Ethanol, Methanol, Acetone and Isopropanol. Removed the use of ethanol only controls. Changed the suggested sequence. Updated the acceptability guidance to be for all the volatile compounds not just ethanol. Added guidance on the reporting of results. (Sections 3.3, 4.0, 5.3, 6.1.3, 6.2, and 6.3)

6	Section 3.3.5 Positive Control changed to Positive Ethanol Control. Section 6.3 Updated reporting to include reporting volatiles and specialized needs of OCME cases.
7	Updated verbiage and formatting within the entire document. Added new set of externally prepared and In-House calibrators and positive control solutions, updated number of positive controls. Updated measurement uncertainty format and explained need for three (3) significant digits within reportable concentrations. Included GC/MS instrumentation and updated GC-FID parameters. Updated the Decision Criteria section to include MS. Updated appendix. Added description of mass spectral comparison. General format rearrangement throughout document.
8	Added title. Updated Sections 1 and 3 with minor edits. Updated formatting throughout document. Updated the note in Section 6 and updated Section 8 to include the AD. Updated Section 6.6. Updated verbiage of Note within Section 7.3 (old Note-05). Updated Section 7.9. Section 9 was revised to remove peak detection and mass spectral comparison criteria as well as Note-08. Removed any indication of use of this SOP with non-biological matrices. Changed ‘correlation coefficient’ definition to the correct term, ‘coefficient of determination.’ Updated Section 7.3 to need Lead Examiner (or higher) approval in order to change sequence steps. Replaced ‘verified’ with ‘validated’ in terms of ensuring quality of reagents/materials prior to use and expiration throughout document. Updated 6.2.1, 6.2.2, 6.2.3, 6.3.1, 6.3.2, 6.3.3 to include handling and expiration information. Instrumental parameters for HS-GC removed since they are no longer necessary. Updated internally-prepared calibrator and positive control solution’s expiration dates from one year to one month.