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 (methanol, acetone and isopropanol) 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.

2. Principle

This procedure involves the analysis of samples 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) or mass spectrometry (MS). 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.

3. Specimens

Any aqueous sample may be suitable for this analysis including, but not limited to: blood, serum, urine, and 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 made 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 0.40 g%). All liquid samples should be prepared using a 1:100 dilution prior to sampling of 200 uL.

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

4. Equipment/Materials/Reagents

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

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

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

4.4 Vortex mixer

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

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

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

4.8 Deionized water (DIW, Millipore, or equivalent)

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

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

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

4.12 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))

5. 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. 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 used
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

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.

<u>Note</u>: Additional positive controls (other than what are listed) and 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.

<u>Note</u>: 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 in the solution preparation log. The appropriate FSE2 (or higher) should be notified just to ensure nothing in procedure will be negatively impacted.

<u>Externally-Prepared Volatile Calibrator Solutions</u>:

5.1 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))

5.1.1 Carefully open (e.g., break top of glass ampoule), individually transfer liquid into autosampler vial, cap, and securely seal. Store refrigerated.
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 ampoules. No dilutions are necessary.

5.1.2 Manufacturer expiration dates are only valid for unopened solutions. When refrigerated, opened solutions are stable for one (1) month from date of opening.

Externally-Prepared Positive Control Solutions:

5.2 Ethanol$_{(aq)}$ [CRM] Positive Control Solution (EtOH$_{(aq)}$; 0.080 g% (w/v)$_{(aq)}$)

5.2.1 Carefully open (e.g., break top of glass ampoule), individually transfer liquid into autosampler vial, cap, and securely seal. Store refrigerated.
The solution should be: 800 μg/mL of EtOH in 1.2 mL ampoules. No dilutions are necessary.

5.2.2 Manufacturer expiration dates are only valid for unopened solutions. When refrigerated, opened solutions are stable for one (1) month from date of opening.

5.3 Externally-Prepared Volatile [CRM] Positive Control Solution (MeOH$_{(aq)}$, EtOH$_{(aq)}$, Acetone$_{(aq)}$, IPA$_{(aq)}$; 0.10 g% (w/v)$_{(aq)}$)

5.3.1 Carefully open (e.g., break top of glass ampoule), individually transfer liquid into autosampler vial, cap, and securely seal. Store refrigerated.

5.3.2 Manufacturer expiration dates are only valid for unopened solutions. When refrigerated, opened solutions are stable for one (1) month from date of opening.

Internal Standard (I.S.) Solutions:

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

5.4.1 Transfer 280 μL of n-propanol into a 2.0 L volumetric flask, dilute to volume with DIW, and mix well.

5.4.2 Transfer to an amber storage container and properly label.

5.4.3 This solution will be tightly capped and can be stored at room temperature. It is stable for one (1) year.

6. Procedure

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

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

6.2.1 Blank DIW (no internal standard)

6.2.2 Negative Control (contains DIW and Internal Standard)

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

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

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

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

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

6.2.8 Blank DIW (no internal standard)

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

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

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

6.2.12 Volatile Positive Control (e.g., External 0.08 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.

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

6.2.14 Blank DIW ; last sample in batch.

<u>Sample Preparation (Automatic Pipetting of Samples, Calibrators, and Controls)</u>:

<u>Pipettor/Diluter Preparation/Priming</u>:

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 working 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 200 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 1 mL of the $NPA_{(aq)}$ (Internal Standard Working Solution) (This may occur automatically depending on automated pipettor programming).

6.9 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.

6.10 Place the probe inside the appropriately labeled autosample 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.

6.13 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:

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

6.15 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.

6.16 Enter the sequence order for the method Batch Table.

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

6.18 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.

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

6.20 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.

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

6.22 Begin the sequence and analyze the samples.

7. Instrumentation

<u>Instrumental Parameters</u>:

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.

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)	7.9 psi	7.9 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	150	–
Scan Speed	781 (N=3) Scan Speed	–

8. 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.

8.1 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 retention time of a chromatographic peak be within ±0.1 minute of the retention time obtained from analysis of a calibrator or positive control.

8.2 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 20% relative to the ion ratios of the 0.050 g% calibrator. If a different calibrator is to be used for ion ratios a unit lead or higher shall be consulted.

8.3 Batch Acceptance

8.3.1 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.

8.3.2 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 without AD approval and documentation explaining why and how such elimination is statistically allowable.

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

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

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

8.3.5 Quantitation of the headspace-GC data may be performed automatically by the software. 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.

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

8.3.7 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.

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

8.3.9 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.

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

8.3.10 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:

8.3.11 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.

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

8.3.13 Calibration Check (EtOH, MeOH, Acetone, IPA): Quantitative results of the Externally-Prepared Volatile Calibrator Solutions [CRM] must be within +/- 20% of its target value. Results which do not meet this criteria will be recorded in the appropriate Quality Control (QC) chart spreadsheet.

8.3.14 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.

8.3.15 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.

8.3.16 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}

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

8.3.17 <u>Column (BAC1) Sample Replicate Evaluation (for EtOH only)</u>:

8.3.17.1

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 is 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.

8.3.18 <u>Dual Column Evaluation</u>:

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

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

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

8.3.19 <u>Reporting Limit (RL)</u>:

8.3.19.1 For quantitative purposes the reporting limit for volatiles will be 0.02 g%. Anything below 0.02 g% will be reported as “Negative”.

8.3.19.2 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).

8.3.19.3 For urine samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.30 g%, appropriate dilutions and re-analyses are necessary if accurate quantitations above that highest calibrator's concentration are needed. 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).

8.3.19.4 For serum samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.34 g%, appropriate dilutions and re-analyses are necessary if accurate quantitations above that highest calibrator's concentration are needed. 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).

8.3.19.5 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.

8.3.19.6 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.

8.3.19.7 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.

8.3.19.8 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.

8.3.19.9 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.

8.3.19.10 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.

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 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.

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 not be reported as listing an uncertainity value 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. Uncertainity value will be available upon request.

Quality Assurance/Quality Control (QA/QC)

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

9.5 Sensitivity: The limit of quantitation (LOQ/LOD) for this procedure is set to the lowest calibrator (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.

9.6 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).

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

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

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

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

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.

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.