

1.0 Introduction

This procedure describes the analysis of samples for volatile compounds, (e.g., methanol, ethanol, acetone, isopropanol, and analogous compounds). Samples are in an aqueous environment, sealed in vials, and analyzed using a Headspace Gas Chromatograph. Volatile components in the heated aqueous solutions reach an equilibrium with the vapor phase in the sealed vial. An aliquot of the vapor phase is analyzed using dual column gas chromatography/flame ionization detection (GC/FID). Volatile components from each column are identified and quantitated based on a calibration using the peak area ratios between analytes and internal standard.

2.0 Specimens

Any aqueous sample may be suitable for this analysis, including (but not limited to) blood, urine, bile, vitreous humor, gastric contents, and tissue homogenates. The preferred method for blood sample collection includes using airtight tubes that contain potassium oxalate and sodium fluoride (aka, "gray-tops"). Other containers for such samples should be within sealed airtight glass or polypropylene containers and should be properly stored. Tissues should be stored frozen until prepared-homogenized, at which time they should be stored refrigerated. Tissue homogenates are normally prepared as a mass-to-volume ratio with deionized water (e.g., 4g tissue with 16 mL of water). Liquid samples should be stored refrigerated. Only blood, serum, and urine samples are suitable for analysis in driving under the influence (DUI) cases under the current statutes and guidelines.

- 2.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case in JusticeTrax.
- 2.2 When not in the sampling or aliquot process, samples in the Toxicology unit will be maintained in locked storage within the Toxicology unit.
- 2.3 When analysis of samples in the Toxicology unit is complete, they must be maintained "Under Proper Seal." Refer to SOP TX-19 for further guidance.
- 2.4 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.
- 2.5 Samples are maintained in the Toxicology Section for a minimum of 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. Samples from fatalities and sexual assaults are maintained indefinitely by the DSS. Cases with requests for retention are maintained by the

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laboratory based on the request. Upon request from the courts or submitting agency the evidence will be returned to the submitting agency.

3.0 Materials, reagents, and Equipment

3.1 Equipment:

HS-GC-1

3.1.1 Shimadzu GC2010 Gas Chromatograph (GC) with a Shimadzu AOC-5000 CTC autosampler for headspace sampling/injection equipped with dual FID detectors, Rtx-BAC1 (Restek 18001 ; 30m, 0.53mm, 3 μ m) and Rtx-BAC2 (Restek 18000 ; 30m, 0.53mm, 2 μ m) capillary columns, or equivalent.

HS-GC-2

3.1.2 Shimadzu GC2010 Gas Chromatograph (GC) with HS 20 autosampler for headspace sampling/injection equipped with dual FID detectors, Rtx-BAC1 (Restek 18003, 30m, 0.32mm, 1.8 μ m) and Rtx-BAC2 (Restek 18002, 30m; 0.32mm, 1.2 μ m) capillary columns, or equivalent.

Note: Pairs of columns can be used with either HS-GC-1 or HS GC-2 instruments.

3.1.3 Automatic pipetter-diluter, or equivalent.

3.1.4 20mL headspace autosample vials with appropriate seals and caps, and crimper.

3.1.5 Class A volumetric glassware

3.1.6 General laboratory glassware and equipment

3.2 Reagents:

3.2.1 Ethanol (EtOH; Baker; anhydrous 200 proof USP or equivalent)

3.2.2 Deionized water (DIW; Millipore or equivalent In-House supply)

3.2.3 Acetone (Baker HPLC grade or equivalent)

3.2.4 n-Propanol (NPA; Baker HPLC grade or equivalent)

3.2.5 Sodium Azide (Baker or equivalent)

3.2.6 Methanol (MeOH; Baker HPLC grade or equivalent)

3.2.7 Isopropanol (IPA; Baker HPLC grade or equivalent)

3.2.8 Ethanol Certified Reference Material (CRM) Standard, Cat E-30 (Cerilliant or equivalent)

3.2.9 Certified Reference Material (CRM) volatile mixture.

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3.3 Preparation of Calibrators, Controls and Standard Stock Solutions:
Check balance using calibrated weights on the day of solution preparation.

3.3.1 Volatile Stock Solution (1.0g% – MeOH, EtOH, Acetone, IPA))

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

3.3.1.2 Properly label the solution.

3.3.1.3 This solution should be stored refrigerated, and should be stable for at least 1 year from the date of validation.

3.3.2 Low Calibrator (0.020g/100mL; 0.02g%)

3.3.2.1 Using a volumetric pipette, add 2mL of the 1.0%(w/v) Volatile Stock solution to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.2.2 Properly label the solution.

3.3.2.3 This solution should be stored refrigerated, and should be stable for at least 1 year from the date of validation.

3.3.3 Medium Calibrator (0.10g/100mL; 0.1g%)

3.3.3.1 Using a volumetric pipette, add 10mL of the 1.0%(w/v) Volatile Standard Stock solution to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.3.2 Properly label the solution.

3.3.3.3 This solution should be stored refrigerated and should be stable for at least 1 year from the date of validation.

3.3.4 High Calibrator (0.30g/100mL; 0.3g%)

3.3.4.1 Using a volumetric pipette, add 30mL of the 1.0%(w/v) Volatile Standard Stock solution to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.4.2 Properly label the solution.

3.3.4.3 This solution should be stored refrigerated and should be stable for at least 1 year from the date of validation.

*Approved by Director: Dr. Guy Vallaro***3.3.5 Positive Control Solution (0.50g/100mL; 0.5g%)**

- 3.3.5.1 Accurately weigh 5.00g acetone, methanol, isopropanol, and ethanol and quantitatively transfer to a 1L volumetric flask with DIW, add 0.2g of sodium azide, dilute to volume with water, and mix well.
- 3.3.5.2 Transfer into a small amber glass bottle and properly label.
- 3.3.5.3 This solution should be stored refrigerated and should be stable for at least 1 year from the date of validation.

3.3.6 Medium Control (0.10g/100mL; 0.1 g%) Volatile Mix

- 3.3.6.1 Using a volumetric pipette, add 10mL of the 1.0%(w/v) Volatile Standard Stock solution to a 100mL volumetric flask, dilute to volume with water, and mix well.
- 3.3.6.2 Properly label the solution.
- 3.3.6.3 This solution should be stored refrigerated and should be stable for at least 1 year from the date of validation.

Note: When preparing calibrators and controls separate stock solution is required.

3.3.8 n-Propanol Internal Standard Stock Solution (5.6g% NPA)

- 3.3.8.1 Transfer 7mL of n-propanol to a 100mL volumetric flask, dilute to volume with water, and mix well. (density of NPA is 0.803g/mL)
- 3.3.8.2 Properly label the solution.
- 3.3.8.3 This solution should be stored refrigerated and should be stable for at least 1 year from the date of validation.

3.3.9 n-Propanol Internal Standard Working Solution (0.011g% NPA)

- 3.3.9.1 Dilute 4.0mL of NPA Internal Standard Stock Solution to a final volume of 2L with DIW and mix thoroughly.
- 3.3.9.2 Transfer into a small amber glass bottle and properly label.
- 3.3.9.3 This solution should be stored refrigerated and should be stable for at least 1 year from the date of validation. Check for bacterial growth prior to use.

3.3.10 Ethanol CRM Standard (aka: Positive Control Solution) (0.08g% EtOH)

- 3.3.10.1 Carefully open ampule containing CRM standard, transfer liquid into autosample vial, and properly label.
- 3.3.10.2 Cap and seal vial after use.

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3.3.11 Volatile Mixture CRM Standard (purchased at a level between 0.01 g% to 0.3 g%)

3.3.12 In lieu of preparing calibrators and or controls CRM can be purchased for this use. Levels should be in the same general range as the prepared solutions (but they need not be the exact same levels).

Note: Expiration date of the CRM check solution in current use, along with the lot number, target value, and acceptable ranges are detailed in Quality Control (QC) charts found within an Excel file on network drive.

3.3.13 Negative Control Solution (0.0g% EtOH)

3.3.12.1 Blank water (this will eventually contain the internal standard)

3.3.12.2 This should be prepared fresh.

3.3.14 Water Blank (no internal standard will be added to this)

3.4 Validation of Reagents:

Validated reagents should be marked with a green dot/sticker detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Reagents can be considered validated when there is acceptable performance of all batch control materials and overall batch acceptability. Preparation of reagents and their validation is documented in the Unit's Reagent Preparation Validation Logbook. This log is maintained in the Toxicology unit.

4.0 Procedure

Sample Preparation

4.1 Pipetter/Diluter - Preparation/Priming:

4.1.1 Turn on the Pipetter/Diluter and check for bacterial contamination in solutions.

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

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

4.1.4. Press the prime switch, liquid will be dispensed from the probe at this time, as the system primes the lines. Cycle until bubbles have disappeared from lines.

4.2 Set-Up for Sample Preparation

- 4.2.1 Scroll to [Run an Existing Method], then press [Select].
- 4.2.2 Highlight [ETHANOL], and then press [Select].
- 4.2.3 The instrument will ask for confirmation of syringe sizes, Press [Confirm].
- 4.2.4 The instrument will ask for initialization, Press [Confirm].
- 4.2.5 Headspace Vial Labeling Note: Samples are run in duplicate.

Label vials for calibrators and controls as follows (suggested working sequence):

- 1. Negative Water Control (containing IS)
- 2. 0.02% Volatile Working Solution (aka: conditioner)
- 3. Low Calibrator (0.02 g/%)
- 4. Medium Calibrator (0.10g/%)
- 5. High Calibrator (0.30g%)
- 6. 0.5g% Positive Control (prepared in duplicate)
- 7. Water Blank
- 8. EtOH CRM Standard (0.08g%)
- 9. Volatile Mix CRM (if to be run)
- 10. $X_1 \dots X_n$;Samples; (each in duplicate)
- 11. After approximately every 10 case sample injections, a QC check solution (Medium Volatile Control (0.10g/%) should be analyzed
- 12. The sequence should end with the Volatile Mix CRM Standard

Note: Each analyst contributing samples to a batch should prepare and analyze their own set of controls and follow the above steps with the exception of preparing and analyzing the calibrator solutions.

4.3 Automatic Pipetting of Samples, Calibrators, and Controls

- 4.3.1 Place the probe in the solution to be sampled and press the Pipette Activation Button (PAB) once to aliquot 1mL of diluent/internal standard solution.
- 4.3.2 Press the PAB again to draw up 200 μ L of the sample, calibrator, or control.
- 4.3.3 Place the probe inside the appropriately labeled autosample vial, and press the PAB once to dispense the aliquots into the autosampler vial.
- 4.3.4 Place the probe in a waste container and press the PAB once more to dispense the between sampling rinse to waste. Ensure there is no carryover of sample. Wipe probe with tissue as needed.
- 4.3.5 Place a headspace cap on the vial and crimp-seal.
- 4.3.6 Proceed and repeat with steps 4.3.1 – 4.3.5 for all calibrators, controls, and samples.
- 4.3.7 Place the labeled vials in appropriate order into the headspace autosampler.

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- 4.3.8 A capped vial containing only water (1mL) should be placed in the autosample holder just after the vial containing the EtOH CRM Standard (0.08g%).
- 4.3.9 These will be verified before and after the analysis.

Note: Analysts will use the EMIT ethanol results (for urine samples) as guidance when considering if the sample needs to be diluted for head space analysis. Samples with an EMIT result of greater than 0.5 will be diluted.

- 4.4 Clean-Up of Automatic Pipetter/Diluter:
 - 4.4.1 Press [Escape] (ESC) from the current method.
 - 4.4.2 Press [ESC] again to get to the main menu.
 - 4.4.3 Press the prime switch. Liquid will be dispensed from the probe at this time.
 - 4.4.4 Place the probe tubing into a waste reservoir and run thru a minimum of 3 cycles.
 - 4.4.5 Return prime switch to original position.
 - 4.4.6 Turn the instrument off and place the probe in the probe holder on the side of the pipetter/diluter.
- 4.5 All biological specimens must be handled with care and are considered biohazards. Universal Precautions for handling biological specimens must be observed at all times and is outlined in the Laboratory Safety Manual.
- 4.6 All specimens and solutions should be removed from the refrigerator and be at room temperature for at least 30 minutes before use in order to ensure that the solutions are visibly clear and free from bacterial growth. If solutions are cloudy or have any signs of contamination, discard the solution(s) and prepare fresh.
- 4.7 If a limited volume of specimen is available for analysis, discuss with the Deputy Director or Lead Examiner in order to determine how to proceed.

5.0 Instrumental Analysis

- 5.1 Gas Chromatograph Setup:
 - 5.1.1 Turn the air and hydrogen tank valves (counterclockwise) for the FID's.
 - 5.1.2 Ramp the column, as needed, at high temperature (200°C) for a short period of time to clean column.
 - 5.1.3 In the GC Solution program which controls the Shimadzu gas chromatograph, enter the sequence order for the method Batch Table and for all urine samples, enter under the dilution factor column 0.769 (this the urine conversion factor).
 - 5.1.4 Save the Batch Table to the day's date and in under the Batch Processing icon, press the Start Icon.

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5.2 Autosampler Configuration:

5.2.1 For the HS-GC-1 instrument, place the vials in the proper order in the Shimadzu autosampler

5.2.1.1 From the touch pad enter the proper "to and from" vial numbers for the AOC Controller.

5.2.1.2 Select start.

5.2.2 For the HS-GC-2 instrument, the vial positions are controlled through the software.

5.2.3 The vial sequence is checked both prior to and after the injection of samples when the auto injector is used. The check after the injection of samples is documented.

5.3 Calibration/Quantitation:

5.3.1 Data reduction of the headspace-GC run for the volatiles is performed by the Shimadzu chemstation using GC Solution software.

5.3.2 Quantitative calculations are based on a comparison of the analyte to internal standard peak area response ratio for the controls and samples, to a linear calibration curve ($y = mx + b$) established from similar ratios from the known calibrator solutions. The method is programmed to produce a linear three-point calibration curve, not including the origin as a point.

5.3.3 Calibration solutions only need to be analyzed once per instrumental sequence.

Instrumental Parameters:

HS-GC(FID) for HS-GC-1:

Temperature program	Alcohols		
Parameter			
Initial temp	50°C		
Ramps:	rate	temp	time
Rate (°C/min), Final temp (°C), Final time (min.)	00.0	50°C	3.4min.*
Post temp	50°C		
Post time (Equil. Time)	0.2 min		
Run Time	3.2 min (3.4min. total)		
Front inlet			
Mode	Split		
Injector temp	200°C		
Pressure	8.6 psig		

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Column/Total flow	12.53 mL/min / 72.7 mL/min
Gas type	Helium
Detector temp	280°C

* GC Run time may be extended when necessary

HS-GC-1: Autosampler Parameters:

AOC-5000	HS-GC-1		AOC-5000	HS-GC-1
Cycle	HS-Inj		Syringe Temp	75°C
Syringe	2.5 mL-HS		Fill Speed	500µL/sec
Sample Volume	850uL		Pull Up Del.	500 msec.
Incubation Temp	50°C		Inject to	GC injector 1
Incubation Time	4 min.		Inject Speed	1mL/sec
Agitation Speed	300 rpm		Pre-Inj Del.	500 msec.
Agitation On	3 sec.		Post Inj. Del	0 msec.
Agitation Off	1 sec.		Syringe Flushing	00:00:25 (25 sec.)
			GC Run Time	00:04:00 (4 min.)

HS-GC(FID) for HS-GC-2:

Autosampler (HS-20)	Parameters		Autosampler (HS-20)	Parameters
Oven Temp	55°C			
Sample Line Temp	150°C		Injection Port SPL1	
Transfer Line Temp	150°C		Injection Mode	Split
Shaking Level	1		Carrier Gas	He
Multi Injection Count	1		Flow Control Mode	Velocity
Multi Injection Count	1		Pressure	136.6 kPa
Pressurizing Gas Pressure	50.0 kPa		Total Flow	35.0 mL/min.
Equilibrating Time	15.00 min.		Column Flow	5.00 mL/min.
Pressurizing Time	0.15 min.		Linear Velocity	65.1 cm/sec.
Pressure Equilib. Time	0.35 min.		Purge Flow	10.0 mL/min
Load Time	0.50 min.		Split Ratio	4.0
Load Equilib. Time	0.25 min.		High Pressure Injection	Off
Injection Time	0.50 min.		Carrier Gas Saver	Off
Needle Flush Time	0.50 min.		Splitter Hold	Off
GC Cycle Time	4.51 min.			
Check GC Ready	On		Column Oven	

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Extended GC Ready Check Limit	10 min.	Initial Temperature	50.0°C
Check System Ready	On	Equilibration Time	0.0 min
Extended System Ready Check Limit	45 min.	Temperature Program	Total Program Time: 4.0 min
Analysis Mode	Constant	Ramp Rate	0°C/min
Needle Check	Yes	Final Temp.	50.0
Action on Leak Check Error	Continue	Hold Time	4.00
Action with No vial on Tray	Skip		

HS-GC-1:

Column Information	Restek, Bellefonte, PA	Restek, Bellefonte, PA
Column Names	Rtx-BAC1	Rtx-BAC2
Column Cat. #	18001	18000
Film Thickness	3.0 µm	2.0 µm
Column Length	30.0 m	30.0 m
Inner Diameter	0.53 mm	0.53 mm
Column Max Temp	260°C	260°C
Detector	FID 1	FID 2
Temperature	280°C	280°C
Sampling Rate	200 msec.	200 msec.
Stop Time	3.2 min. (+ 0.2min. Equil)	3.2 min. (+ 0.2min. Equil)
Delay Time	0.00 min	0.00 min
Makeup Gas	He	He
Makeup Flow	30.0 mL/min	30.0 mL/min
H ₂ Flow	40.0 mL/min	40.0 mL/min
Air Flow	400.0 mL/min	400.0 mL/min

HS-GC-2:

Column Information	Restek, Bellefonte, PA	Restek, Bellefonte, PA
Column Names	Rtx-BAC1	Rtx-BAC2
Column Cat. #	18003	18002
Film Thickness	1.80 µm	1.20 µm
Column Length	30.0 m	30.0 m
Inner Diameter	0.32 mm	0.32 mm
Column Max Temp	260°C	260°C
Detector	FID 1	FID 2

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Temperature	260°C	260°C
Sampling Rate	40 msec.	40 msec.
Stop Time	4.00 min	4.00 min
Delay Time	0.00 min	0.00 min
Subtract Detector	None	None
Makeup Gas	He	He
Makeup Flow	30.0 mL/min	30.0 mL/min
H2 Flow	40.0 mL/min	40.0 mL/min
Air Flow	400.0 mL/min	400.0 mL/min

Data processing:

GC Solution software Batch List		
Dilution Factor	0.769 (for urine)	0.862 (serum)

6.0 Decision Criteria**6.1 Run Acceptance Criteria:**

- 6.1.1 Run Completion: The batch must have been injected with no unexplained interruption and no unexplained errors. If unsure whether to continue, consult the Deputy Director or lead Examiner.
- 6.1.2 Blank and Carryover Check: No significant integrated peaks (other than the internal standard) should be noted in the negative control sample. Any automatically integrated target analytes present in the blanks or negative control samples should be at concentrations below 0.005g% EtOH.
- 6.1.3 Calibration Check (Accuracy & Precision): Results for the EtOH CRM Standard must be within 5% of its target 0.08% value, range 0.076 – 0.084%. Results are recorded and documented in the Excel QC charts.
- 6.1.4 Calibration Linearity: The calibration curve is considered acceptable if the correlation coefficient (r^2) is ≥ 0.99 . The calibration curve for each analyte is independently evaluated.
 - 6.1.4.1 If the calibration curve is not acceptable, the run is rejected and case samples will not be analyzed until a calibration curve is acceptable.
 - 6.1.4.2 Instrument troubleshooting and/or preparation of new calibration solutions, if necessary, should be undertaken prior to analysis of samples.
 - 6.1.4.3 Results are recorded and documented in the Excel QC charts.

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- 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.
 - 6.1.4.5 If the positive control results are outside the designated acceptable limits, the casework samples that were analyzed prior to and after the positive control will be evaluated and may need to be re-analyzed. Consult Unit Lead when necessary.
 - 6.1.4.6 If the positive control results are outside the designated acceptable limits, appropriate corrective action may need to be done before casework samples are analyzed. Consult Unit Lead when necessary.
- 6.2 Sample Acceptance Criteria:
- 6.2.1 The peaks of interest should show good chromatographic peak shape, symmetry, width, and resolution. In order to be determined acceptable, a chromatographic peak should compare favorably to that of the same analyte in the positive control which was analyzed contemporaneously.
 - 6.2.2 Relative retention times (compared to the internal standard) for any identified analyte must be within 0.1 minutes of the corresponding relative retention time for the analyte in the control solution.
 - 6.2.3 Any reportable analyte must be able to be identified by retention time on both columns and the quantitative values from both columns for that analyte must agree within 10%.
 - 6.2.4 The difference between the BAC1 column results for each replicate should be $\leq 5\%$ when the analyte value is $\geq 0.08g\%$.
 - 6.2.5 The difference between the BAC1 column results for each replicate should be $\leq 10\%$ when the analyte value is between $0.03g\%$ and $0.08g\%$.
 - 6.2.6 The difference between the BAC1 column results for each replicate can be $\geq 10\%$ when the analyte value is $\leq 0.03g\%$.
 - 6.2.7 If the above criteria are not met, a unit Lead or higher will be consulted and the samples may need to be repeated. If blood specimens are clotted or the difference between the 2 EtOH results is greater than 5%, the samples should be centrifuged and analyzed as serums.
 - 6.2.8 Urine samples with converted ethanol equivalent of $> 0.38g\%$ must be reanalyzed using appropriate dilutions.
 - 6.2.9 Blood samples with ethanol concentrations $> 0.5g\%$ must be re-analyzed utilizing appropriate dilution(s).

6.2.10 Samples with volatile results (other than ethanol) that are outside the calibration range will be assessed to determine if they need to be diluted and repeated. The Unit Lead or Deputy Director should be consulted for these cases.

6.2.11 Samples which have analytes that elute after a GC run has completed (i.e., over 4 minutes) may need to be reanalyzed using a longer GC run time. Subsequently, sample data that contain carry-over peaks (i.e., the next sample) may also need their samples to be re-analyzed. Consult Unit Lead when necessary.

6.3 Reporting of Results:

6.3.1 EtOH levels < 0.01g% will be reported as "Ethanol Not Identified" (or similar language).

6.3.2 For samples where ethanol is identified, quantitative values ≥ 0.01 g% EtOH should be reported as follows:

6.3.2.1 The reported values will be an average of the two BAC1 column replicate values and they will be truncated to two decimal digits.

6.3.2.2 Urine samples will be converted to whole blood equivalent ethanol values using a conversion factor of 1.3 to 1.

6.3.2.3 Serum samples will be converted to whole blood equivalent ethanol values using a conversion factor of 1.16 to 1.

6.3.6 Ethanol concentration values within non-biological samples will be calculated by weight (i.e., g%) using this procedure. Reporting an ethanol concentration within non-biological samples will involve converting the weight concentration value to a volume concentration value using a density (0.789 g/mL) factor (i.e., v/v % and/or proof).

6.3.7 In samples where Acetone, Methanol and/or Isopropanol is identified the value will be reported using the guidance of 6.3.2.1 - 6.3.2.3 above.

6.3.8 In samples where one or multiple (but not all) volatiles are identified "No other toxicologically significant volatiles were identified" (or similar) will be reported.

6.3.8 In samples where no volatiles are identified "No toxicologically significant volatiles were identified" (or similar) will be reported.

6.3.9 Uncertainty of Measurement will be reported based on the current calculated uncertainty.

7.0 Quality Assurance/Quality Control

7.1 Run and Sample Evaluation, Operator and Analytical Review: Each sample is evaluated for acceptability according to the sample and run acceptance criteria specified above.

7.2 Sensitivity: The limit of detection (LOD) for this procedure has been administratively set at 0.01g% EtOH.

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- 7.3 Specificity: Because of the dual column concept, no potentially interfering substances have been identified using this procedure. All samples are analyzed on two separate columns using differing polarities.
- 7.4 Accuracy: Accuracy of the method is checked using positive controls in addition to a CRM.
- 7.5 Precision: Precision of the method is evaluated for each result and documented within the Excel QC charts.
- 7.6 Linearity: Linearity of the calibration curve for the range of 0.01g% EtOH to 0.3g% EtOH is evaluated on each instrument sequence.
- 7.7 Verification of vial sequence: All vial sequences are checked both prior to and after analyses.

8.0 References

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- 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

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Acetone, Isopropanol and Methanol are all calculated in the same manner

1. Calculate I.S. Ratio:

$$\text{I.S. Ratio} = \frac{\text{Vol. Cal. Analyte Area}}{(\text{I.S. Area}) * 0.1\%}$$

2. Calculate Analyte Concentration:

$$\text{Analyte Concentration} = \frac{\text{Sample Analyte Area}}{\text{I.S. Ratio}}$$

Example: Acetone 0.1% Control:

$$\text{I.S. Ratio \%} = (555008/120851)/0.1 = 45.92$$

$$\text{Control Concentration \%} = (520629/115328)/45.92 = 0.0983$$

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Approved by Director: Dr. Guy Vallaro

Rev. #	Issue Date	History
4	4/14/16	Updated verbiage and formatting within the entire document. Appendices were updated with correct information and formatting was changed. Added 'Blank Water' (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.
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)