1.0 Introduction

This procedure describes the analysis of aqueous samples for volatile compounds, (e.g., methanol, ethanol, acetone, isopropanol, and analogous compounds), utilizing a headspace gas-chromatographic method. Samples are in an aqueous environment, sealed in vials, and analyzed using a headspace analysis. 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. Technical reviews are performed by an Examiner other than the analyst who prepared the samples. Quantitative results from analyses passing the sample acceptance criteria may be reported as follows:

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. 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. Tissue homogenates are normally prepared as a mass-to-volume ratio with deionized water (e.g., 4g tissue with 16mL of water).

2.1 All evidence transfers, either between individuals or between an individual and a storage location, must be recorded (either electronically or by hardcopy). If the chain-of-custody document is in the form of a hardcopy, it will be maintained within the case file.

2.2 When not being actively analyzed, samples will be stored in a secure evidence area (e.g., lockable refrigerator, freezer)

2.3 Samples will be stored under proper seal (i.e., in such a manner so that they are protected from sample loss, cross-contamination, or deleterious change). Examples of proper seal include the use of evidence tape (with initials half on and half off of the tape), a storage box containing an tamper-evident security tag (with initials on the tag), or a plastic bag containing a heat-seal with initials over such seal.

ARCHIVED

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, 3µm) and Rtx-BAC2 (Restek 18000 ; 30m, 0.53mm, 2µm) 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, 1.8µm) and Rtx-BAC2 (Restek 18002, 30m; 0.32mm, 1.2µm) 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 aluminum 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)

ARCHIVED

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

3.3.1 EtOH Standard Stock Solution (1.0g% (w/v))

3.3.1.1 Accurately weigh 5.000g (+/- 0.0025g) of anhydrous EtOH, 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 (i.e., appropriate identification and safety labels (analyte, concentration, date prepared, lot #, validation date and preparer's initials).

3.3.1.3 Document preparation on a "Calibration/Control Standard Preparation Form," and file in the Standards Preparation Logbook.

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

3.3.2 Volatile Stock Solution (1.0g%(w/v) – (MeOH, EtOH, Acetone, IPA))

3.3.2.1 Accurately weigh 5.000g (+/- 0.0025g) 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.

3.3.2.2 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 Low EtOH Calibrant (0.020g/100mL EtOH, aka. 0.02g%)

3.3.3.1 Using a volumetric pipette, add 2mL of the 1.0%(w/v) EtOH 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 Medium EtOH Calibrant (0.10g/100mL EtOH, aka. 0.1g%)

3.3.4.1 Using a volumetric pipette, add 10mL of the 1.0%(w/v) EtOH 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.

ARCHIVED

3.3.5 High EtOH Calibrant (0.30g/100mL EtOH, aka. 0.3g%)

3.3.5.1 Using a volumetric pipette, add 30mL of the 1.0%(w/v) EtOH standard stock solution to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.5.2 Properly label the solution.

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 EtOH Positive Control Solution (0.50g/100mL EtOH, aka. 0.5g%)

3.3.6.1 Weigh 5.00g of ethanol, quantitatively transfer to a 1L volumetric flask with DIW, add 0.2g of sodium azide, dilute to volume with water, and mix well.

3.3.6.2 Transfer into a small amber glass bottle and properly label.

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

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

3.3.7.1 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)

3.3.7.2 Properly label the solution.

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

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

3.3.8.1 Dilute 4.0mL of NPA Internal Standard Stock Solution to a final volume of 2L with DIW and mix thoroughly.

3.3.8.2 Transfer into a small amber glass bottle and properly label.

3.3.8.3 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.

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

3.3.9.1 Carefully open ampule containing CRM standard, transfer liquid into autosample vial, and properly label.

3.3.9.2 Cap and seal vial after use.

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) ethanol charts found within an Excel file on network drive.

ARCHIVED

3.3.10 Volatile Working Solution – (MeOH, EtOH, Acetone, IPA)

3.3.10.1 For 0.02% controls, use a volumetric pipette and add 2mL of the Volatile Standard Stock Solution (1.0g%) to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.10.2 For 0.1% controls, use a volumetric pipette and add 10mL of the Volatile Standard Stock Solution (1.0g%) to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.10.3 For 0.25% controls, use a volumetric pipette and add 25mL of the Volatile Standard Stock Solution (1.0g%) to a 100mL volumetric flask, dilute to volume with water, and mix well.

3.3.10.4 Other solutions of varying concentrations may be made and used. Follow similar process as above steps.

3.3.10.5 Transfer to a brown bottle, label, and store refrigerated. These solutions should be stable for at least one year from the date of validation.

3.3.10.6 This solutions can be used as calibrators or controls, as necessary.

3.3.11 Negative Control Solution (0.0g% EtOH)

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

3.3.11.2 This should be prepared fresh.

3.4 Validation of Reagents:

Validated reagents should be marked with a green dot, 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 can be maintained in the laboratory.

ARCHIVED

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:

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

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 200µL 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 4.3.1.1 – 4.3.1.5 for all calibrators, controls, and samples.
4.3.7 Place the labeled vials in appropriate order into the headspace autosampler.

ARCHIVED

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.

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.

5.2 Autosampler Configuration:

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

ARCHIVED

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 ethanol 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 Ethanol 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.

ARCHIVED

Instrumental Parameters:

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

Temperature program	Alcohols		
Parameter			
Initial temp	50°C		
Ramps: Rate (°C/min), Final temp (°C), Final time (min.)	rate	temp	time
	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		
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	850µL		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**	
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			

ARCHIVED

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
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

ARCHIVED

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 targeted value. 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 $\geq$ 0.99.

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.

6.1.4.4 Precision: All positive control solution quantitation results must agree within 5%.

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 supervisor or lead Examiner 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 supervisor or lead Examiner 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

ARCHIVED

compare favorably to that of the same analyte in the positive control which was analyzed contemporaneoously.

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 ≤ 5% when the ethanol value is ≥ 0.08g%.

6.2.5 The difference between the BAC1 column results for each replicate should be ≤ 10% when the ethanol value is between 0.03g% and 0.08g%.

6.2.6 The difference between the BAC1 column results for each replicate can be ≥ 10% when the ethanol value is ≤ 0.03g%.

6.2.7 If the above criteria are not met, a lead Examiner 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 centrifuged and analyzed as serums.

6.2.8 Samples with ethanol concentrations >0.5g% must be re-analyzed utilizing appropriate dilution(s).

6.2.9 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 supervisor or lead Examiner when necessary.

6.3 Reporting of Results:

6.3.1 For ethanol biological samples only, values ≥ 0.01 g% EtOH should be reported.

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

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

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

6.3.5 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 (0.786 g/mL) factor (i.e., v/v % and/or proof).

ARCHIVED

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.

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

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

ARCHIVED

Appendix

Volatile Manual Calculation without software

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:

I.S. Ratio % = (555008/120851)/0.1 = 45.92

Control Concentration % = (520629/115328)/45.92 = 0.0983

ARCHIVED

Rev. #	Issue Date	History
4	Insert Effective Date	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.

ARCHIVED