1.0 PRINCIPLE: This procedure describes the analysis of aqueous samples for volatile compounds, (methanol, ethanol, acetone, isopropanol, and analogous compounds), utilizing a headspace gas-chromatographic method. Samples are diluted with an aqueous solution containing n-propanol as an internal standard, and sealed in vials for headspace analysis. Volatile components in the heated aqueous phase diffuse into, and reach equilibrium with the vapor phase. An aliquot of the vapor phase is injected into the gas chromatograph (simultaneously onto two separate columns), which separates the analytes as a function of their chemical characteristics. Separated components from each column are identified by retention time, and quantitated by response on Flame-Ionization Detectors. Quantitation is based on a three-point calibration using the peak area ratio between the analyte and the internal standard.

2.0 SPECIMEN: Samples requiring analysis for ethanol and other volatile compounds have their associated case jacket kept in the Toxicilogy lab. 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 should be in airtight tubes containing potassium oxalate and sodium fluoride ("gray-tops"). All other samples should be sealed and stored in appropriate airtight glass or polypropylene containers. Tissues should be stored frozen until homogenization (see Note 2; below) and analysis. If not analyzed immediately, preserved liquid samples should be refrigerated and may be stored (sealed) for up to 12 months. (However, there is no expectation that, in the presence of a patent seal, that there will be degredation of alcohol in a biological sample contained in a gray-top tube, even well beyond 12 months.)

Note 1: Only Blood, Serum and Urine are suitable for analysis in DUI cases under the DESPP guidelines.
Note 2: Tissue homogenates are normally prepared as a 1:5 v:v ratio with DIW (Deionized water); e.g. 4 g tissue + 16 ml DIW.

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, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

2.2 When not in the sampling or aliquot process, samples in the toxicology section must be stored in a secure and locked area.

2.3 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.

2.4 When samples are finished being analyzed, samples in the toxicology section must be maintained "Under Proper Seal." This is interpreted to mean that the sample, or a container in which the

sample is kept is sealed with tamper-evident tape with the initials and date of the person placing the seal clearly marked.

3.0 MATERIALS AND EQUIPMENT:

3.1 Equipment:

3.1.1 Gas Chromatograph with autosampler for headspace sampling/injection; equipped with dual FID detectors, Rtx-BAC1 and Rtx-BAC2 30m capillary columns. (0.53 x 3 um; Restek 18000 & 18001 or equivailent).

3.1.2 Automatic Pipetter-Diluter (200 microliter & 2 ml syringes).

3.1.3 20 ml headspace autosampler vials with appropriate seals and aluminum caps, and crimper.

3.1.4 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 Aqueous EtOH Certified Reference Standard. (Cerilliant or equivailent)

3.3 Preparation of Calibrators, Controls and Standard Stock Solutions:

(Balance used for the preparation of solutions must be checked by calibrated weights on the day of solution preparation)

3.3.1 **1% EtOH Standard Stock Solution**

3.3.1.1 Accurately weigh 5.000 g (+/- 0.0025 g) of anhydrous EtOH, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW, Add 0.1 g of Sodium Azide, Q.S. with DIW and mix well.

3.3.1.2 Label the standard with the appropriate identification and safety labels (Analyte, concentration, preparer, date prepared, 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 bestable for at least 1 year from date of validation.

3.3.2 **1% Acetone Standard Stock Solution**

3.3.2.1 Accurately weigh 5.000 g (+/- 0.0025 g) of Acetone, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW. Add 0.1 g of Sodium Azide. Q.S. with DIW and mix well.

3.3.2.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and should be stable for at least 1 year from date of validation.

3.3.2 **1% MeOH Standard Stock Solution**

3.3.3.1 Accurately weigh 5.000 g (+/- 0.0025 g) of Methanol, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW. Add 0.1 g of Sodium Azide. Q.S. with DIW and mix well.

3.3.3.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and should be stable for at least 1 year from date of validation.

3.3.4 **Isopropanol Standard Stock Solution**

3.3.4.1 Accurately weigh 5.000 g (+/- 0.0025 g) of IPA, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW. Add 0.1g of Sodium Azide. Q.S. with DIW and mix well.

3.3.4.2 Label the standard and document preparation above. This solution should be stored refrigerated, and should be stable for at least 1 year from date of validation.

3.3.5 **Low Cal. (0.02 g/100ml EtOH,)**

3.3.5.1 Using a class A volumetric pipette, add 2 ml of the 1% EtOH stock solution to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

3.3.5.2 Label the standard and document preparation above. This solution should be stored refrigerated, and should be for at least 1 year from date of validation.

3.3.6 **Med Cal. (0.10 g/100ml EtOH.)**

3.3.6.1 Using a class A volumetric pipette, add 10 ml of the 1% EtOH standard stock solution to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

3.3.6.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and should be stable for at least 1 year from date of validation.

3.3.7 **High Cal. (0.30 g/100ml EtOH.)**

3.3.7.1 Using a class A volumetric pipette, add 30 ml of the 1% EtOH standard stock solution to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

3.3.7.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and should be stable for at least 1 year from date of validation.

3.3.8 **0.50g/100 EtOH In-House Control**

3.3.8.1 Weigh 5.00 g of ethanol, quantitatively transfer to a one liter class A volumetric flask with DIW. Add 0.2 g of sodium azide to the volumetric flask. Q.S. with DIW and mix well.

3.3.8.2 Decant contents of the flask into a small amber glass bottle, label appropriately. This solution should be stored refrigerated, and should be stable for at least one year from date of validation.

3.3.9 Internal Standard Stock (nPA) Solution

3.3.9.1 Transfer ~ 7 ml of NPA to a 100 ml volumetric flask. Q.S. with DIW and mix well.

3.3.9.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and should be stable for at least one year from date of validation.

3.3.10 Internal Standard Working Diluent Solution

3.3.10.1 Dilute 4.0 ml of NPA Stock Solution to a final volume of 2000 ml with DIW, mix thoroughly. Decant the contents of the 2000 ml flask into a large, amber bottle.

3.3.10.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and should be stable for at least one year from date of validation.

3.3.11 **Cerilliant Certified Reference Standard Solution**

3.3.11.1 Store original container in the refrigerator until needed. Open ampule containing CRM standard, transfer to GC/MS Vial and properly label. Seal GC/MS vial after use.

Note: Expiration date of the CRM check solution in current use, along withthe lot number, bottle number, target value and acceptable ranges are detailed ~~on page 2 of the Volatile Batch Summary Review Form.~~ in Excel QC ethanol charts.

3.3.12 **Volatile Calibrator Solution**. (0.1%)

3.3.12.1 Using a class A volumetric pipette, add 10 ml each of the 1% MeOH, IPA and Acetone standard stock solutions to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

3.3.12.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and should be stable for at least one year from date of validation.

3.3.13 **Volatile Control Solutions**.

3.3.13.1 For 0.1% controls, use a class A volumetric pipette, add 10 ml each of the 1% MeOH, IPA and Acetone standard stock solutions to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion. For 0.02% controls, use a class A volumetric Pipette, add 2 ml each of 1% MeOH, IPA and Acetone standard stock solutions to a class A volumetric flask. For the 0.3% controls, use a class A volumetric pipette, add 30 ml each of 1% MeOH, IPA, and Acetone standard stock solutions to a class volumetric flask. Q.S. all with DIW and mix well by inversion.

3.3.13.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and should be stable for at least one year from date of validation.

3.3 Validation of Reagents:

Validated reagents are 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. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, Maintained in the Toxicology laboratory.

4.0 PROCEDURE; Sample Preparation

Note 1: All biological specimens must be handled with care, and considered as Bio-Hazardous; "Universal Precautions" for handling biological specimens must be observed at all times, as outlined in the Laboratory Safety Manual.

Note 2: All access to lab specimens being analyzed under CTDESPP guidelines, and alcohol PT samples, must be detailed and documented on the appropriatein of custody (COC) forms.

Note 3: Prior to the withdrawal of aliquots, Cerilliant Calibration Check Solution, Calibrator, Control, Samples and Internal Standard solutions should be removed from the refrigerator, and allowed to stand at room temperature for at least 30 min.

Note 4: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be raised with Quality Manager and/or the Director. If the proposed change will not present a change of a magnitude that would require validation, the change may be approved, and the Director will modify and re-issue the SOP accordingly.

Any such procedural changes would be subject to the review process afforded by the quality control measures of the analytical scheme described herein. Hence, any modification or change that produces an unexpected deleterious effect on the analytical procedure would be expected to trigger analysis or batch failure in the QC review stages.

Note 5: If a limited volume of sample is available for analysis, discussion with director or supervisor should occur to determine what Toxicological procedures should be done, and in what order.

4.1 Pipetter/Diluter - Preparation/Priming:

4.1.1 Turn on the Pipetter/Diluter.

4.1.2 Place the inlet tubing in the NPA diluent 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: ~~All controls and s~~Samples are run in duplicate. Label vials for calibrators and controls as follows:

1 System Conditioner (any calibrator or control)
2 Low Calibrator 1 (0.02 g/%)
3 Medium Calibrator 1 (0.10 g/%)
4 High Calibrator 1 (0.30 g%)
5 0.5 g% EtOH In-house Control
6 Blank DI Water 1 carry over check
7 CER; CRM Rep 1
8 Volatile (any calibrator or control)
9 $X_i \ldots X_n$;Samples; (each in duplicate)
10 After every 10 injections from case samples run 0.10 g% EtOH In-house Control
11 etc.
12 Bracket casework with a 0.10 g% EtOH In-house Control
13 End with CER; CRM Rep 2

~~System conditioner (any level calibrator or control) (1)~~
~~Low Calibrator (1)~~
~~Medium Calibrator (1)~~
~~High Calibrator (1)~~

~~Negative control (1)~~
~~Cerilliant(certified reference material) (2)~~
~~In House 0.5% Control Ethanol (2)~~
~~In House 0.1% Control Ethanol (2)~~
~~Volatile Calibrator (1)~~
~~Volatile Control (2)~~
~~Samples 2 (up to 15 cases) (2 vials/submission).~~
~~In House Control Ethanol -2~~

Note: Each analyst contributing samples to a batch must prepare an additional set of controls
~~**Note**: For every 10 cases, another set of ethanol controls (2) must be prepared.~~Prepare enough controls to bracket all casework. i.e . Every ten case sample injections

4.3 Preparation of Calibrators, Cerilliant and Controls

4.3.1 Automatic Pipetting of Samples, Calibrators and Controls

4.3.1.1 Place the probe in the solution to be sampled, and Press the Pipette Activation Button (PAB) once to aliquot 1 ml of diluent/internal standard solution.

4.3.1.2 Press the PAB again, to draw up 200 µl of the sample, calibrator or control.

4.3.1.3 Place the probe inside the appropriately labeled autosampler vial, and press the PAB once to dispense the aliquots into the autosampler vial.

4.3.1.4 Place the probe in a waste container, and press the PAB once more to dispense the between sampling rinse to waste.

4.3.1.5 Place a headspace cap on the vial and crimp - seal.

4.3.1.6 Proceed with steps 4.3.1 – 4.3.5 for all calibrators, controls, and samples.

4.3.1.7 Repeat for all calibrators, controls and samples.

4.3.1.8 Place the vials sequentially into the sampling carriage Headspace Autosampler in the sequence detailed below:

Sample #	Contents
1	System Conditioner (any calibrator or control)
2	Low Calibrator 1 (0.02 g/%)
3	Medium Calibrator 1 (0.10 g/%)
4	High Calibrator 1 (0.30 g%)
5	0.5 g% EtOH In-house Control
6	Blank DI Water 1 carry over check
7	CER; CRM Rep 1
8	Volatile (any calibrator or control)

9 $X_i \ldots X_n$;Samples; (each in duplicate)
10 After every 10 injections from case samples run 0.10 g% EtOH In-house Control
11 etc.
12 Bracket casework with a 0.10 g% EtOH In-house Control
13 End with CER; CRM Rep 2
~~7 CER; CRM Rep 2~~
~~8 0.5 g% EtOH In-house Control Rep1~~
~~9 0.5 g% EtOH In-house Control Rep2~~
~~10 0.1 g% EtOH In-house Control Set 1 Rep 1~~
~~11 0.1 g% EtOH In-house Control Set 1 Rep 2~~
~~12 Volatile Calibrator 1~~
~~13 Volatile Control Rep 1~~
~~14 Volatile Control Rep 2~~
~~15 $X_i \ldots X_n$;Samples; (each in duplicate)~~
~~X_n+1 0.1 g% EtOH In-house Control Set 1 Rep 1~~
X_n+2 0.1 g% EtOH In-house Control Set 1 Rep 2

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 3 cycles.
4.4.5 Return prime switch to original postion.
4.4.6 Turn the instrument off and place the probe in the probe holder on the side of the pipetter/diluter.

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 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.
5.1.3 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 Place the vials in the proper order in the Shimadzu autosampler and from the touch pad

enter the proper "to and from" vial numbers for the AOC Controller. Select start.

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. Quantitative calculations are based on a comparison of the analyte to I.S. 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, including the origin as a point. Each headspace run is independently calibrated. The calibration curve is considered acceptable if the correlation coefficient (r^2) is $\geq$ 0.99. If not, the run is rejected. Corrective action, including instrument troubleshooting and/or preparation of new calibration solutions should be undertaken prior to repeat analysis of samples. Other volatiles, acetone, methanol, isopropanol, are not usually reported. ~~are calculated by hand using a one point calibration and high and low controls of 0.02% and 0.3% for Methanol, Isopropanol, and Acetone. See appendix III for sample calculations.~~

~~5.4 Batch Summary Sheets are prepared for each batch (See Appendix II).~~

6.0 RUN EVALUATION

6.1 Run Acceptance Criteria

6.1.1. Run Completion: The batch must have been injected with no unexplained interruption, and no instrumental unexplained errors. If unsure whether to continue, consult Director or Supervisor.

6.1.2 Blank and Carryover Check: No significant integrated peaks (other than the internal standard) should be noted in the blank sample. Any target analytes present in the Carryover check must be at concentrations below the maximum allowable (0.005 g%). ~~Both checks are documented on p. 2 of the Batch Summary Form.~~

6.1.3 Calibration Check (Accuracy & Precision): EtOH results for both replicates of the Certified Reference Material solution must be within 5% of the target value.~~, as detailed on the Volatile Batch QC Review Form (page 2). Replicates must agree within 5%, and are similarly documented.~~ Results are recorded and documented it the QC charts.

6.1.4 Calibration Linearity: The correlation coefficient of the calibration curve must be greater than or equal to 0.99. ~~, and is documented on page 2 of the Batch Summary Form.~~

6.1.5 Accuracy: 0.5 g% EtOH Control (Accuracy & Precision): EtOH results for both replicates (per set, if applicable) of the In-House 0.1, and 0.5 g% control solutions must be within 10% of their target value. ~~, as detailed on the "Volatile Batch QC Review Form (page 2).~~ Results are recorded and documented it the QC charts.

6.1.6 Precision: All Control duplicate quantitative ~~(Column A)~~ analysis results must agree within 5%.

6.1.7 If the run is rejected, corrective action, including instrument troubleshooting, proper documentation and/or preparation of new calibration solutions should be undertaken prior to repeat analysis of samples. A QAR may be initiated, depending on the specific failure issue.

6.2 Sample Acceptance Criteria

6.2.1 Chromatography must be acceptable for all reported analytes in sample chromatograms, on the quantitation column.

6.2.2 Relative retention times for any identified analyte in control or samples must be within 0.1 min of the corresponding retention time for the analyte in the calibration solution. (This is automatic, as a function of the instrument qualitative ID window.)

6.2.3 Any reportable analyte must have been identified by the software by retention time on both columns, and the quantitative values must agree within ~~20%~~ 10%. ~~, as detailed on page 1 of the Batch Summary Form.~~

6.2.4 Duplicate quantitative results for any reportable analyte must be within 5% of each other, The difference between the BAC1 column results must be less than or equal to 5% when the alcohol value is ≥ 0.08%. Results that are <0.08 and ≥ 0.05 must be less than 10%. Results that are <0.05 should have a difference of less than 0.01g%. If the appropriate results are not met, the sample must be repeated. If the blood specimens are clotted or the difference between the 2 results is greater than 5% the samples should be run as serums. ~~as detailed on page 1 of the Batch Summary Form (with the exception of low level autopsy cases, per analyst discretion).~~

6.2.5 DUI Samples with Ethanol concentrations > 0.5 g% must be re-analyzed with dilution. Post-mortem samples with such concentrations may be accepted at the discretion of the Director.

6.2.5.1 Samples needing dilution should be diluted with DIW (e.g. 100 uL sample, + 100 uL DIW, thoroughly mixed), with the dilution volumes, and appropriate multiplier documented in the case record, GC sample information, and GC batch documents.

6.3 Analytical Review; A Technical Review of the batch is performed by an analyst other than the batch operator, checking the run and sample acceptance criteria as noted above, and ensuring correct transcription of GC data onto ~~the batch summary forms~~ QC charts. Reviews are documented. ~~on the batch summary forms.~~

6.4 **Reporting of Results;** Quantitative Results from analyses passing the sample acceptance criteria described above may be reported as follows: Only values ≥ 0.01 g/% are reported, with the ~~lower value~~ average of the two BAC1 replicate analyses ~~from the "A" column being reported~~, truncated to two decimal digits being reported

Urine Samples are required to be reported in terms of blood equivalent results. This laboratory uses a conversion factor of 1.3 to 1. This is done by entering 0.769 in the dilution factor column in the GC Solution software Batch List. Serum samples use a conversion factor of 1.16 to 1. This is done by entering 0.862 in the dilution factor column in the GC Solution software Batch List.

Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).

7.0 **Quality Assurance/Quality Control**

7.1 **Run and Sample Evaluation, Operator and Analytical Review;** Each run is evaluated according to both the Sample and Run Acceptance Criteria specified above. Run acceptance is documented by both the operator, and a second, independent reviewer. Similarly, each sample is evaluated for acceptability according to the Sample acceptance Criteria specified above.

7.2 **Report Administration and Final Review:**

7.2.1 Prior to any result being issued, each case file is subjected to an technical and administrative review (in accordance with ASCLD guidelines) to ensure that appropriate documentation is present in the file, that results were generated from appropriately reviewed and accepted analytical batches, and that reported results have been correctly transcribed.~~from batch summary sheets and final reports~~

7.2.2 Prior to the final sign-off, each case is subjected to a final review by the Director or designee. This review is designed to ensure that appropriate testing has been done, and that the results in the case file have been generated in a forensically defensible manner.

7.3 **Sensitivity:** Sensitivity of the method has been documented by performance on the external PT program (CAP, NHTSA, ODOH) between the ranges of 0.02 g/% - 0.50 g/%. (will be 0.5%)

7.4 **Specificity:** Specificity of the method has been documented by performance on the external PT program and the volatile controls, containing potentially interfering substances. All samples are analyzed on two separate columns, of differing polarity. No substances interfering with any target analyte at the appropriate retention time on both columns have been observed to date.

7.5 **Accuracy:** Accuracy of the method is checked on each batch by the analysis of Certified Reference material, in addition to the control materials.

7.6 **Precision:** Precision of the method is evaluated for each result and documented on the ~~batch summary spreadsheet~~QC charts. Precision of reported values in ensured by the requirement that any reportable result agree with its replicate analysis to within 5%.

7.7 **Linearity:** Linearity of the Calibration Curve for the range of 0.0 - 0.3 g/100 ml is evaluated on each instrument run, and is required to be > 0.99 (r**2).

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7.8 **Performance Testing Samples:** This laboratory participates in outside Proficiency Testing Programs; **College of American Pathologists- Alcohol Proficiency** and **DOT- Alcohol Proficiency**

Results from this proficiency-testing program is reviewed upon receipt. Any significant problem with a PT result is addressed by the Chief Toxicologist/Laboratory Director. Any such problems and corrective/remedial action are documented in the PT notebook for the department.

7.9 Verification of Vial Sequence: 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 on the run summary sheet.

7.10 ~~The Batch Summary Sheets are reviewed with e~~Each batch is reviewed~~,~~ by the analyst and Technical Reviewer. Both analyst and reviewer ~~reviews~~ are documented on the ~~Summary Sheets~~ QC charts.~~, and~~ Standards, controls, and blanks for the batch are stored in the toxicology lab. ~~maintained with batch GC Data~~

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

Appendix I:
GC/Headspace general temperature program specifications

Temperature program	Alcohols

Parameter			
Initial temp	50° C		
Ramps Rate/final temp/final time	rate	temp	time
	00.0	50	3.4
Post temp	50°c		
Post time	0.00		
Run Time	3.4 min		
Front inlet			
Mode	split		
Initial temp	200°c		
Pressure	8.6 psig		
Total flow			
Gas type	Helium		
Injection volume	650 uL		
Detector temp	300°c		
Plenum temp	70°c		
Equilibrium time	4 min		
Syringe temp	80 c		
Injection Speed	1 ml/s		
Agitation Speed	300 rpm		

~~Appendix II:~~

~~Example of controlled Alcohol batch document- TX ALCOHOL-1. Batch documents can vary based on nature of batch.~~

Volatile Batch Summary

CT Forensic Toxicology Laboratory
Batch ID: 1-4-11

Analyst(s): _____

Sample	Matrix	Ethanol Conc. Gm % Col. A Rep 1	Col. A Rep 2	Col. B Rep 1	Col. A Avg.	Col. A % Diff.	B:A Delta %	Other Volatiles: (Note units)
Conditioner	Water	0.0269		0.0234				
.02 Cal	Water	0.0200		0.0200				
0.10 Cal	Water	0.1000		0.1000				
0.3 Cal	Water	0.3005		0.3004				
Blank	Water	0.0000		0.0000				
CRM 0.08 Control	Water	0.0783	0.0790	0.0789	0.0787	-0.89	-0.32	
0.10 Control	Water	0.0977	0.0975	0.0988	0.0976	0.20	-1.23	
vol cal	Water	0.0000	0.0000	0.0000	0.0000	#####	######	
vol control	Water	0.0000	0.0000	97.0000	0.0000	#####	######	
TX-10-1921-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	Acetone was detected
TX-10-1921-2	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	Acetone was detected
TX-10-1922-1	Urine	0.2537	0.2536	0.2560	0.2537	0.04	-0.93	
0.10 Control	Water	0.0970	0.0973	0.0989	0.0972	-0.31	-1.80	
TX-10-1923-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1923-2	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1927-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1927-2	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1928-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1928-2	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1929-1	blood	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1929-2	blood	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1931-1	Urine	0.0094	0.0094	0.0096	0.0094	0.00	-2.13	
TX-10-1942-1	Urine	0.1604	0.1612	0.1627	0.1608	-0.50	-1.18	
TX-10-1943-1	Urine	0.0641	0.0653	0.0747	0.0647	-1.85	-15.46	
0.10 Control	Water	0.0972	0.0969	0.0989	0.0971	0.31	-1.91	
TX-10-1948-1	blood	0.2848	0.2976	0.2852	0.2912	-4.40	2.06	
TX-10-1949-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1952-1	blood	0.1726	0.1724	0.1759	0.1725	0.12	-1.97	
TX-10-1952-2	blood	0.1562	0.1459	0.1610	0.1511	6.82	-6.59	Repeat
TX-10-1953-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1956-1	blood	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1956-2	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
TX-10-1957-1	Urine	0.0000	0.0000	0.0000	0.0000	#####	######	
0.10 Control	Water	0.0975	0.0981	0.0987	0.0978	-0.61	-0.92	

Acceptable Limits: +/- 5% +/- 20%

Analyst Run Review: Run Acceptable? _____ Date: _____
Comments: _____ _____
_____

Vial position verified prior to sample removal: _____

Analytical Review: Run Acceptable?:_____

NOT NEEDED Delete this form

~~Example of controlled Alcohol batch document TX ALCOHOL-2. Batch documents can vary based on nature of batch.~~

Department of Public Safety
Forensic Toxicology Laboratory

Batch ID: ___3-8-11_________

Volatile Batch Summary / Review Form - Page 2

Note: Run Review / Acceptance Documented on Page 1

Each Volatile Batch is independently calibrated for EtOH at 0.020, 0.100 and 0.300 g/100 ml. Validity of the Ethanol calibration is demonstrated by analysis of Certified Reference Material (Guth), and the correlation coefficient of the best-fitting straight line. Validity of other analyte calibration is demonstrated by acceptable control performance.

Ethanol Calibration Linearity: Accepted?
EtOH Calibration Correlation Coefficient (>/= 0.99): ☐ Yes ☐ No

Ethanol Carryover Check (Blank, Position 5): Accepted?
Blank Sample, Following High Cal. (</= 0.005): _______ ☐ Yes ☐ No

External Certified Reference Material (NIST-Traceable)
0.08 Cerilliant Solution; ETOH-80 Expiration Date: 09/1/12 Lot#: FN092407-01
Target Value: 0.0801 g/% (Acceptable Range = Target Value +/- 5%)
Published Value: Initials:
Acceptable Range: 0.0761 to 0.0841 g/% Accepted?
Rep. 1 Result: ____________ ☐ Yes ☐ No
Rep. 2 Result: ____________ ☐ Yes ☐ No

In-House, 0.1 g/% Control (1 set per analyst):
Target Value: 0.1000 g/% (Acceptable Range = Target Value +/- 10%)
Acceptable Range: 0.09 to 0.11 g/% Accepted?

EtOH Control Set 1 Rep 1: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 2: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 1: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 2: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 1: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 2: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 1: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 2: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 1: ____________	☐ Yes	☐ No	Analyst: ____________
EtOH Control Set 1 Rep 2: ____________	☐ Yes	☐ No	Analyst: ____________

Batch Review Documentation: Batch Accepted?
Analyst: __________________ Date: ____________ ☐ Yes ☐ No ☐ Partial
QC Reviewer: __________________ Date: ____________ ☐ Yes ☐ No ☐ Partial

Analyst Notes: ____________________________________

QC Reviewer Notes: ____________________________________

NOT NEEDED Delete this form

Appendix III

~~Non-Ethanol~~ Volatile Manual Calculation without software.

Note: 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