Raleigh/Wake City-County
Bureau of Identification
Crime Laboratory Division

Drug Chemistry Unit
BLOOD CHEMISTRY TECHNICAL PROCEDURES

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1: Technical Procedure for Quality Assurance

1. **Purpose / Scope** – This procedure provides direction for the receipt and quality assurance of laboratory supplies, equipment, reagents, reference collections, reference standards and reference materials that affect casework in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification Crime Laboratory

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.2. Performance verification – The initial confirmation of the reliability of a previously or externally validated method or instrument.
   2.3. Commercial reagent - A purchased solvent or chemical.
   2.4. Critical reagent - Chemicals or reagents which critically affect the quality of tests which do not have their reliability verified as part of the quality control checks in a DWI Blood Chemistry Unit Technical Procedure.
   2.5. Prepared reagent - Mixture of two or more reagents or a dilution.
   2.6. Reference standard - Measurement standard designated for the calibration of other measurement standards (reference standards or equipment.)
   2.7. Reference material - Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.
   2.8. Primary reference material - Any reference material obtained from a commercial source which has documentation issued by the manufacturer certifying its chemical composition or has documentation stating the manufacturer's specifications for the material. This material may be certified reference material if available and practicable.
   2.9. Secondary reference material - Reference material from a non-commercial source or from a commercial source which does not have authenticating documentation from the manufacturer or is derived from reference material.
   2.10. Authenticating documentation - A certificate of analysis provided by the manufacturer certifying chemical composition or a statement of the manufacturer's specifications or any published spectral data from an informed treatise generally accepted in the field that identifies a chemical substance.
   2.11. Purchasing documentation – Any requisition forms, vendor quotes and packing slips associated with the purchase and receipt of Drug Chemistry Unit laboratory supplies, equipment, reagents, reference collections, reference standards and reference materials.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. GC-MS – gas chromatograph-mass spectrometer
   3.3. MS – mass spectrum
   3.4. QCC – quality control check

3.5. RRT – relative retention time

4. Procedure for Laboratory Supplies and Commercial Reagents

4.1. Prior to use, received laboratory supplies and commercial reagents shall be inspected for compliance with specifications as stated in the purchasing documentation. The purchasing documentation shall reflect any specifications required in the applicable DWI Blood Chemistry Unit Technical Procedure.

4.2. When laboratory supplies are found to meet specifications they shall be stored for use in the DWI Blood Chemistry Unit. When commercial reagents are found to meet specifications they shall be marked with the initials of the receiving Blood Drug Chemist and the date received. Commercial reagents shall be stored in accordance with the manufacturer's specifications, if applicable, and in accordance with the CCBI Crime Laboratory Safety Manual.

4.2.1. If applicable, a copy of the packing slip shall be marked with the initials of the receiving Blood Drug Chemist and the date of receipt.

4.2.2. The requisition shall be marked with the date of receipt and the initials of the receiving Blood Drug Chemist.

4.2.3. Forward the original completed requisition to the Deputy Director and maintain a copy in the DWI Blood Chemistry Unit along with any other associated purchasing documentation.

4.3. Laboratory supplies and commercial reagents that do not meet specifications shall be not be used. They shall be stored in a separate area and clearly marked "Not for Use" until they can be returned or otherwise disposed.

4.3.1. Mark the packing slip, if applicable, and the requisition form with the discrepancy, initials of the receiving Blood Drug Chemist and forward to the Forensic Quality Manager and Deputy Director. Maintain a copy in the DWI Blood ~~Drug~~ Chemistry Unit along with any other associated purchasing documentation.

4.4. Upon being opened, commercial reagent containers shall be marked as opened along with the initials of the Blood Drug Chemist and the date.

4.4.1. When a commercial reagent is transferred to another container it shall be labeled with the following:

4.4.1.1. Identity and grade, if applicable
4.4.1.2. Supplier and lot number
4.4.1.3. Initials of the Blood Drug Chemist
4.4.1.4. Date
4.4.1.5. Expiration date, if applicable.

5. Procedure for Prepared Reagents

5.1. Reagents may be prepared in any amount provided that the component ratios in the DWI Blood Chemistry Unit Technical Procedure are kept constant.

5.2. Labeling

5.2.1. Lot numbers for containers of prepared reagents shall be assigned using lot number designations as specified in the DWI Blood Chemistry Unit Technical Procedure.

5.2.2. Containers of prepared reagents shall be labeled with the following:

5.2.2.1.1. Identity of the reagent
5.2.2.1.2. Initials of preparer
5.2.2.1.3. Date of preparation
5.2.2.1.4. Lot number
5.2.2.1.5. Expiration date
5.2.2.1.6. QCC due date

5.2.3. Each new container of prepared reagent shall be documented in the reagent log with the following:

5.2.3.1.1. Identity of the reagent
5.2.3.1.2. Lot number
5.2.3.1.3. Reference to the DWI Blood Chemistry Unit Technical Procedure followed for preparation
5.2.3.1.4. Initials of preparer
5.2.3.1.5. Date of preparation
5.2.3.1.6. Expiration date
5.2.3.1.7. QCC result and supplier and lot number of any reference material used
5.2.3.1.8. Component(s) and supplier and lot number

5.3. Storage

5.3.1. Reagents shall be stored in closed containers and, if applicable, as specified in the DWI Blood Chemistry Unit Technical Procedure used for preparation.

5.4. Expiration Dates

5.4.1. Internal standard solutions, calibration solutions and verification solutions shall expire one year after preparation unless otherwise specified in the DWI Blood Chemistry Unit Technical Procedure used for preparation. All other prepared reagents shall expire three years after preparation unless otherwise specified in the DWI Blood Chemistry Unit Technical Procedure used for preparation.

5.5. Quality Control Checks

5.5.1. Prepared reagents shall be quality control checked according to the DWI Blood Chemistry Unit Technical Procedure used for preparation prior to or concurrent with their initial use.

5.5.2. Prepared reagents, other than internal standard solutions, calibration solutions and verification solutions, with an expiry greater than six months must be have QCC(s) repeated every six months to ensure reagent reliability.

5.5.3. Document quality control checks in the reagent log with the following:

5.5.3.1. Date performed
5.5.3.2. Initials of Blood Drug Chemist
5.5.3.3. Reference material, supplier and lot number
5.5.3.4. QCC result
5.5.3.5. Due date for next QCC

6. Procedure for Reference Materials

6.1. Reference material containers shall be received and labeled by the Blood Drug Chemist as directed for commercial reagents, refer to Section 4.

6.1.1. In the event that the container is too small to be labeled as required, the reference material may be stored in a larger container or the required information may be recorded in the reference material log. Blood Drug Chemists shall refer to the reference material log for any information not recorded on the container prior to using reference material.

6.2. Prior to use, each new lot of reference material used in the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography shall be analyzed according to that procedure to ensure that the materials are appropriately identified and suitable for use.

6.3. Prior to use, each new lot of reference material, other than those used in the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography, shall be analyzed by mass spectrometry, at a minimum, using DWI Blood Chemistry Unit Technical Procedures to ensure that the materials are appropriately identified and suitable for use.

6.3.1. The Blood Drug Chemist shall qualitatively evaluate the data produced. The reference material must be found to be substantially comparable to the authenticating documentation provided by the supplier, if applicable, and substantially comparable to authenticating documentation from a source other than the supplier, reference material, or published spectral libraries. Reference material that does not meet these requirements shall not be used.

6.4. Reference material found to be suitable for use shall be marked "APD" along with the date, Blood Drug Chemist initials and the QCC due date, refer to 6.4.1. All data and authenticating

documentation shall be marked by the Blood Drug Chemist with initials and date and maintained in the DWI Blood Chemistry Unit.

6.4.1. The reference material evaluation must be repeated after one year if the reference material is to be used for identification of a substance or in a QCC.

6.5. Reference materials shall be maintained in room C1387, C2427 or C1403 and stored according to the manufacturer's instructions, if applicable.

6.6. A reference material log shall be maintained.

6.6.1. Each Blood Drug Chemist who uses reference materials shall update the reference material log with initials and date. For solid material include the gross weight of reference material prior to removing material for use, the amount removed and the gross weight of reference material as returned to storage. For liquids include only the volume removed.

6.7. An audit of the DWI Blood Chemistry reference materials shall be conducted annually according to the CCBI Administrative Procedure for Annual Quality Audits.

7. Procedure for Critical Reagents

7.1. Negative blood

7.1.1. Negative blood shall be received and labeled by the Blood Drug Chemist as directed for commercial reagents, refer to Section 4, and stored in room C1387 or C2427 in the freezer. A container may be thawed and divided into smaller containers for individual use. Mark the smaller containers with the original lot number followed by a unique, sequential letter. Once placed in the refrigerator for thawing, the individual container shall be marked with an expiry of one month.

7.1.1.1. In the event that the container is too small to be labeled as required, the ~~reference~~ material may be stored in a larger container or the required information may be recorded in a Critical Reagent logbook. Blood Drug Chemists shall refer to the Critical Reagent logbook for any information not recorded on the container prior to using the ~~reference~~ material.

7.1.2. Prior to use, for each new lot of negative blood a single sample must be analyzed according to the DWI Blood Chemistry Unit Technical Procedures for Enzyme Linked Immunosorbent Assay (ELISA) as a Drug Screen, ~~Solid Phase Extraction of Basic Drugs for GC-MS Analysis~~, Solid Phase Extraction of ~~Acidic / Neutral~~ Drugs for GC-MS Analysis (analysis of basic and acidic/neutral fractions), Solid Phase Extraction of Benzodiazepines for GC-MS Analysis and Solid Phase Extraction of THC and THC-COOH for GC-MS.

7.1.2.1. The negative blood must not contain any controlled substances or controlled substance metabolites. The presence of any non-controlled substances identified, e.g., caffeine, shall be recorded.

7.1.3. Negative Blood found to be suitable for use shall be marked "APD" along with date and initials. All analysis data and authenticating documentation shall be marked by the Blood Drug Chemist with initials and date and maintained in the Critical Reagent logbook in the DWI Blood Chemistry Unit.

7.2. Chemical derivatizing agents

7.2.1. Chemical derivatizing agents shall be received and labeled by the Blood Drug Chemist as directed for commercial reagents, refer to Section 4, and stored in room C1387, C1403, or C2427 according to the manufacturer's instructions, if applicable. Once opened, the container shall be used and disposed on the same day.

7.2.1.1. In the event that the container is too small to be labeled as required, the ~~reference~~ material may be stored in a larger container or the required information may be recorded in a Critical Reagent logbook. Blood Drug Chemists shall refer to the Critical Reagent logbook for any information not recorded on the container prior to using the ~~reference~~ material.

7.2.2. Prior to use, for each new lot of chemical derivatizing agent, morphine reference material must be derivatized and analyzed by GC-MS.

7.2.2.1. The derivatized morphine mass spectrum must be substantially the same as reference material.

7.2.3. Chemical derviatizing agents found to be suitable for use shall be marked "APD" along with date and initials. All analysis data and authenticating documentation shall be marked by the Blood Drug Chemist with initials and date and maintained in the Critical Reagent logbook in the DWI Blood Chemistry Unit

8. Procedure for In-house Generated Reference Collections

8.1. Spectral and relative retention time reference collections generated within the Laboratory will be traceable to primary reference materials, if practicable, otherwise secondary reference materials may be used. Data and authenticating documentation shall be maintained in the DWI Blood Chemistry Unit.

8.2. The DWI Blood Chemistry Unit GC-MS Relative Retention Time reference collection shall also include the LOD, LOQ and calibration limits for ethanol, methanol, isopropanol, acetone, THC and THC-COOH.

8.3. When reference collections are updated they shall be renamed to include the date of revision. The previous version shall be archived. Current and archived in-house generated spectral reference collections shall be maintained by the DWI Blood Chemistry Technical Leader.

9. Procedure for Reference Standards

9.1. Refer to the Drug Chemistry Unit Technical Procedure for Balances

10. Safety

10.1. Refer to the CCBI Crime Laboratory Safety Manual

11. Records

11.1. Reagent log

12. References

12.1. Mills, III, Terry and Roberson, Conrad J., *Instrumental Data for Drug Analysis*, 2nd Ed., Vols. 1-5, CRC Press, Inc., 1993.

12.2. Moffat, Jackson, Moss and Widdop**,** *Clarke's Isolation and Identification of Drugs*, 4th Ed., 2011.

12.3. Pfleger, Maurer, and Weber, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*; 2nd. Ed., Vols. 1-3, 2007.

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3, updated lines 6.5. and 6.6.1.
1/16/15	2	Update and additions to 7.1, 7.1.1.1, 7.1.2, 7.2.1, 7.2.1.1 and 8.2.

2: Technical Procedure for Uncertainty of Measurement

1. **Purpose / Scope** - This procedure is utilized to estimate the uncertainty of measurement for test methods for which a numerical value is reported on a Laboratory Report in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification Crime Laboratory.

2. **Definitions**

   2.1. Uncertainty of measurement - a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand.
   2.2. Coverage probability (Level of confidence) - probability that the set of true quantity values of a measurand is contained within a specified coverage interval.
   2.3. Coverage factor - numerical factor used as a multiplier of the combined uncertainty in order to obtain an expanded uncertainty.

3. **Abbreviations**

   3.1. UOM – uncertainty of measurement
   3.2. CU – combined uncertainty
   3.3. EU – expanded uncertainty

4. **Procedure**

   4.1. The DWI Blood Chemistry Technical Leader shall determine an estimation of the UOM for each test method for which a numerical value is reported on a laboratory report. The specific measuring device or instrument used for a reported test result must be evaluated in the estimation of the UOM for that test method.

   4.2. The estimation of the UOM shall be performed annually, at a minimum, or when a change in measurement conditions occurs that may have a significant effect on the UOM.

   4.3. Laboratory environmental conditions shall be monitored and any additional effect on UOM shall be evaluated upon collection of data. Refer to the DWI Blood Chemistry Unit Technical Procedure for General Laboratory Equipment.

   4.4. Each test method requiring UOM shall be evaluated for contributions from sources of uncertainty, u. The contributions shall be evaluated using Type A methods (by a statistical analysis of measured values obtained under defined measurement conditions such as repeatability and / or reproducibility, including measurement assurance data) and Type B methods (by other means of analysis of components from such things as instrument readability, calibration certificate reported uncertainty, etc.)

4.5. Evaluate the identified sources of uncertainty and combine them to obtain the combined uncertainty of measurement, CU, using the formula

$CU = \sqrt{(u_1^2 + u_2^2 + u_3^2 \ldots)}$
where
CU = combined uncertainty
u_1, u_2, etc. = individual identified sources of uncertainty

4.6. The combined uncertainty of measurement is an estimation of the uncertainty of measurement, UOM. Individual sources of uncertainty that are not significant contributors may be excluded.

4.7. The expanded uncertainty, EU, shall be calculated to provide a minimum 99.73 % coverage probability (or approximately 99%) by multiplying the CU by the appropriate coverage factor, k.

4.8. Round the EU to two significant digits. Do not perform rounding prior to this step.

4.8.1. When the digit next beyond the one to be retained is less than five, keep the retained figure unchanged. For example: 2.541 becomes 2.5 to two significant figures.

4.8.2. When the digit next beyond the one to be retained is greater than five, increase the retained figure by one. For example: 2.453 becomes 2.5 to two significant figures.

4.8.3. When the digit next beyond the one to be retained is exactly five, and the retained digit is even, leave it unchanged; conversely if the digit is odd, increase the retained figure by one (even/odd rounding). Thus, 3.450 becomes 3.4 but 3.550 becomes 3.6 to two significant figures.

4.8.4. When two or more figures are to the right of the last figure to be retained, consider them as a group in rounding decisions. Thus, in 2.4501, the group (501) is considered to be greater than 5 while for 2.5499, (499) is considered to be less than 5.

4.9. For blood alcohol and acetone determination, multiply the % EU by the average of the four measured values (gram / 100 ml), to obtain an EU expressed in the same units as the measurement result.

4.10. The reported EU shall contain at most two significant digits and be reported to the same level of significance as the measurement result. Any requirements for the level of significance for reporting of the measurement result shall also determine the level of significance for reporting the EU. The reported EU shall be rounded, refer to 4.8.1 – 4.8.4 for rounding.

4.10.1. For blood alcohol and acetone determination, the blood alcohol concentration is truncated to the hundredths place according to North Carolina General Statutes § 20-4.01.(1b)a. and the associated EU is rounded to the hundredths place.

4.11. The EU shall be reported for each test method where a numerical value is reported on a laboratory report. When numerical results are added to produce a combined result the respective EU's shall also be added.

4.12. The laboratory report shall identify the measured quantity value, *y*, along with the associated EU. The result shall be reported as $y \pm EU$, with the units of *EU* consistent with the units of *y*. The coverage probability shall be included.

4.12.1. Examples:

4.12.1.1. Ethanol only identified:

The blood alcohol concentration is 0.10 gram per 100 milliliters ± 0.01 gram per 100 milliliters of whole blood at a coverage probability of 99.73%.

The blood alcohol concentration is 0.37 gram per 100 milliliters ± 0.02 gram per 100 milliliters of whole blood at a coverage probability of 99.73%.

4.12.1.2. Ethanol and Isopropanol identified:

The blood alcohol concentration is X.XX gram per 100 milliliters ± X.XX gram per 100 milliliters of whole blood at a coverage probability of 99.73%.
The ethanol concentration is X.XX gram per 100 milliliters ± X.XX gram per 100 milliliters of whole blood at a coverage probability of 99.73%.
The isopropanol concentration is X.XX gram per 100 milliliters ± X.XX gram per 100 milliliters of whole blood at a coverage probability of 99.73%.

4.13. Additionally, for blood alcohol and acetone determination, round the EU determined in 4.9 to a maximum of two significant figures and round the average of the measured values to the same level of significance (decimal places). Refer to 4.8.1 – 4.8.4 for rounding.

4.13.1. Add an additional statement containing the values from 4.13 to the report.

4.13.1.1. *Example:*

(The average of the four measured alcohol concentrations is 0.1034 gram per 100 milliliters ± 0.0062 gram per 100 milliliters of whole blood at a coverage probability of 99.73%.)

(The average of the four measured alcohol concentrations is 0.374 gram per 100 milliliters ± 0.022 gram per 100 milliliters of whole blood at a coverage probability of 99.73%.)

(The average of the four measured alcohol concentrations is 0.0123 gram per 100 milliliters ± 0.0007 gram per 100 milliliters of whole blood at a coverage probability of 99.73%.)

4.14. When compliance with a statutory limit is in question, ~~i.e.,~~ such as 0.01, 0.04, 0.08 and 0.15 gram of alcohol per 100 milliliters of whole blood, add an additional statement to the report to clearly communicate this information.

4.14.1. *Example:*

Taking the estimated measurement uncertainty into consideration, there is a possibility that the blood alcohol concentration is less than 0.08 gram per 100 milliliters of whole blood.

4.15. The DWI Blood Chemistry Unit Technical Leader shall maintain records of the estimation of the uncertainty of measurement in the DWI Blood Chemistry Unit. The records shall:

- Define the measurand
- State how traceability is established for the measurement
- State the equipment (measuring device(s)) used
- State all uncertainty components considered
- Identify all uncertainty components of significance and how they were evaluated
- Contain the data used to estimate repeatability and / or reproducibility
- Contain all calculations performed
- State the combined standard uncertainty, CU, the coverage factor, k, the coverage probability, C, and the resulting expanded uncertainty, EU
- The minimum due date for the review/recalculation of the measurement uncertainty, refer to 4.2.

5. Calculations

5.1. $CU = \sqrt{(u_1^2 + u_2^2 + u_3^2 \ldots)}$

5.2. Average or mean, $\bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i$

6. References

6.1. *ASCLD/LAB Level 100A Traceability presentation*, Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

6.2. *ASCLD/LAB Level 100B Measurement Assurance presentation*, Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

6.3. *ASCLD/LAB Level 100C Measurement Uncertainty Concepts presentation*, Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

6.4. *ASCLD/LAB Level 200 Measurement Confidence for the Forensic Laboratory: Measurement Uncertainty in Drug Chemistry presentation*, Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

6.5. *ASCLD/LAB Level 200 Measurement Confidence for the Forensic Laboratory: Measurement Uncertainty in Toxicology Testing presentation***,** Copyright 2011; Heusser Neweigh, LLC & ASCLD/LAB.

6.6. *Introduction to Measurement Uncertainty course***,** LeBeau, Marc A., Ph.D., 2009, RTI International

6.7. *Introduction to Measurement Uncertainty – Practical Examples Part II course***,** LeBeau, Marc A., Ph.D., 2010, RTI International

6.8. *Introduction to Measurement Uncertainty – Practical Examples Part III course***,** LeBeau, Marc A., Ph.D., 2010, RTI International

6.9. *Evaluation of measurement data - Guide to the expression of uncertainty in measurement, JCGM 100:2008 GUM 1995 with minor corrections***,** First edition September 2008**,** JCGM 2008**,** Working Group 1 of the Joint Committee for Guides in Metrology (JCGM/WG 1)

6.10. *ASCLD/LAB Policy on Measurement Uncertainty,* ASCLD/LAB, AL-PD-3060 Ver 1.1.

6.11. *ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – Overview,* ASCLD/LAB, AL-PD-3061 Ver 1.0.

6.12. *ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX A, Details on the NIST 8-Step Process*, ASCLD/LAB, AL-PD-3062 Ver 1.0.

6.13. *ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX D, Toxicology Testing Discipline Example – Concentration of Ethanol in an Ante-Mortem Blood Specimen*, ASCLD/LAB, AL-PD-3065 Ver 1.0.

6.14. *GLP 9 Good Laboratory Practice for Rounding Expanded Uncertainties and Calibration Values,* National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, February 2012.

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3.
12/16/13	2	Compliance with new ASCLD/LAB policies
4/14/14	3	Add report statement for compliance with statutory limits
10/1/14	4	Removed rounding in 4.9
1/16/15	5	Corrected typographical error in 4.8.2. Added clarification in 4.13 and example in 4.13.1.1. Added additional examples of statutory limits in 4.14.

3: Technical Procedure for General Laboratory Equipment

1. **Purpose / Scope** - This procedure provides direction for the use of general laboratory equipment in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.2. Performance verification – The initial confirmation of the reliability of a previously or externally validated method or instrument.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. NIST – National Institute of Standards and Technology
   3.3. QCC – Quality control check

4. **Equipment, Materials**

   4.1. Equipment

      4.1.1. Refrigerator
      4.1.2. Freezer
      4.1.3. Eppendorf Reference Mechanical Pipettes and corresponding pipette tips
      4.1.4. Mettler XS1003S Balance
      4.1.5. Class A volumetric glassware, pipettes and volumetric flasks
      4.1.6. Nichiryo Accupenser Junior Dispensers
      4.1.7. Thermometers, NIST traceable
      4.1.8. Laboratory environmental monitor – barometer, hygrometer, thermometer, NIST traceable
      4.1.9. Millipore Elix Advantage Water Purification System

   4.2. Materials

      4.2.1. Weighing vessels
      4.2.2. Deionized water
      4.2.3. Millipore Elix Advantage Progard Pack
      4.2.4. Millipore Elix Advantage Reservoir Vent Filter
      4.2.5. Millipore Elix Advantage UV 254 lamp

4.2.6. Millipore Elix Advantage chlorine tablet

4.3. Reference Standards

4.3.1. NIST traceable weights: 0.1 g, 2 g, 200 g

5. Procedure for Refrigerators and Freezers

5.1. Monitor the temperature of each refrigerator used for storage of evidence, chemicals or reference materials each working day.

5.2. Record the temperature in degrees Celsius on the refrigerator or freezer temperature log.

5.3. The acceptable temperature for refrigerators is 4^0C ($\pm$ 3^0C). The acceptable temperature for freezers is -15^0C or below.

5.3.1. If the temperature is unacceptable adjust the temperature setting.

5.3.2. Notify the Blood Drug Chemistry Technical Leader for scheduling of service or replacement if the temperature cannot be corrected.

5.3.2.1. Mark improperly functioning evidence refrigerators "out of service," relocate the evidence and record in the case record

5.4. Record all adjustments, maintenance and comments in the refrigerator or freezer maintenance log.

5.5. Maintain refrigerator and freezer logs in the DWI Blood Chemistry Unit.

6. Procedure for Mechanical Pipettes

6.1. Mechanical pipettes shall be certified by the manufacturer and appropriate for the intended use.

6.2. After one year of service the mechanical pipettes shall be serviced and calibrated annually by an approved vendor.

6.3. Perform performance verification on all mechanical pipettes used for evidence handling, i.e., 1 ml and 0.25 ml pipettes, prior to their initial use. Label the pipettes "Blood." Follow the procedure for a monthly quality control check, refer to 6.4.1.

6.4. Perform a quality control check monthly on all "Blood" mechanical pipettes.

6.4.1. Monthly Quality Control Check

6.4.1.1. Inspect the pipette and tips for proper functioning, refer to 11.1.

6.4.1.1.1. If a pipette problem is observed, remove the pipet from service, label it "out of service," and notify the DWI Blood Chemistry Technical Leader for scheduling of repair.

6.4.1.1.2. If pipette tip problems are observed, segregate the pipette tips and clearly label "not for use." Notify the DWI Blood Chemistry Technical Leader.

6.4.1.2. Allow the pipettes, pipette tips, deionized water and environmental monitor to equilibrate in the measurement location for a minimum of two hours at a temperature between 20 and 25 ^{0}C $\pm$ 0.5^{0}C.

6.4.1.3. Record the temperature and air pressure using the environmental monitor.

6.4.1.4. Place the pipette tip on the tip cone of the pipette.

6.4.1.5. Fill the weighing vessel with deionized water to a minimum height of 3 millimeters.

6.4.1.6. Fill the pipette tip and empty five times with the deionized water.

6.4.1.7. Discard the pipette tip and replace.

6.4.1.8. Pre-wet the tip one time.

6.4.1.9. Hold the pipette vertically.

6.4.1.10. Dip the pipette tip into the deionized water by a few millimeters.

6.4.1.11. Draw in the volume to be tested slowly and evenly and wait three seconds.

6.4.1.12. Pull the pipette tip slowly out of the liquid, wiping it on the vessel wall.

6.4.1.13. Rest the filled tip up against the wall of the weighing vessel at an angle.

6.4.1.14. Dispense the test liquid slowly until the first stop (measuring stroke).

6.4.1.15. Press the control button to the second stop (blow-out) and dispense the remaining liquid in the tip.

6.4.1.16. Hold down the control button and pull the tip up the vessel wall.

6.4.1.17. Let the control button slide back into position.

6.4.1.18. Determine and record the weight.

6.4.1.19. Repeat nine times.

6.4.1.20. Calculate the systematic error in percent.

6.4.1.20.1. Calculate the mean value, $\bar{x}$, of the dispensed volume, using the correction factor, z, refer to page 29 of reference 11.2.

$$\bar{x} = z\left(\frac{\sum All\ measured\ values}{n}\right)$$

where n = number of measured values

6.4.1.20.2. Calculate the systematic error, e_s, in percent.

$$e_s = 100\left(\frac{\bar{x} - x_{nominal}}{x_{nominal}}\right)$$

where $x_{nominal}$ = target volume

6.4.1.20.3. The acceptable range is ∓ 0.9 %.

6.4.1.21. Calculate the random error as coefficient of variation.

6.4.1.21.1. Calculate the repeat standard, s, using the correction factor, z, refer to page 29 of reference 11.2

$$s = \sqrt{\frac{\sum(x_i z - \bar{x})^2}{n-1}}$$

6.4.1.21.2. Calculate the coefficient of variation, CV.

$$CV\ (\%) = 100\frac{s}{\bar{x}}$$

6.4.1.21.3. The acceptable range is ∓ 0.4 %.

6.4.1.22. Record the systematic and random error on the Pipette Monthly Quality Control Check form.

6.4.1.23. If an error is outside of the acceptable range, the QCC may be repeated. If the problem persists, remove the pipet from service, label it "out of service," and notify the DWI Blood Chemistry Technical Leader for scheduling of repair.

6.5. Prior to each use ensure that the monthly QCC, if applicable, has been successfully performed, refer to 6.4.

6.6. Prior to each use inspect the pipette and tips to ensure that the control button is moving properly, a visually adequate volume of liquid is aspirated and delivered, there is no dripping.

6.6.1. If a pipette problem is observed, remove the pipet from service, label it "out of service," and notify the DWI Blood Chemistry Technical Leader for scheduling of repair.

6.6.2. If a pipette tip problem is observed, segregate the pipette tips and clearly label "not for use." Notify the DWI Blood Chemistry Technical Leader.

6.7. Pipette operation

6.7.1. Pre-wet pipette tips by aspirating and dispensing the liquid to be measured a few times before pipetting. With the tip not in contact with the liquid, empty it completely on the inner wall of the tube (via blow-out).

6.7.2. Aspirating

6.7.2.1. Attach corresponding pipette tip to pipette.
6.7.2.2. Press down the control button to the first stop (measuring stroke).
6.7.2.3. Immerse the pipette tip vertically approximately 3 millimeters into the liquid.
6.7.2.4. Allow the control button to slide back slowly.
6.7.2.5. Pull the tip out of the liquid slowly.
6.7.2.6. To remove any droplets, dab with line-free wipe, ensuring that no liquid comes out of the tip.

6.7.3. Dispensing

6.7.3.1. Hold the tip at an angle against the inside wall of the tube.
6.7.3.2. Press down the control button slowly to the first stop (measuring stroke) and wait until the liquid stops flowing.
6.7.3.3. Press down the control button to the second stop (blow-out) until the liquid stops flowing.
6.7.3.4. Hold down the control button and pull the tip up the inner wall of the tube.
6.7.3.5. Eject tip by pressing the control button to the final stop.

6.8. Record maintenance and service in a log maintained in the DWI Blood Chemistry Unit.

6.9. Maintain manufacturer's certificates, performance verification documentation, quality control check documentation and calibration certificates in the DWI Blood Chemistry Unit.

7. Procedure for Balance

7.1. Balances shall be certified by the manufacturer and appropriate for the intended use.

7.2. After one year of service balances shall be serviced and calibrated annually by an approved vendor.

7.3. Perform performance verification on all balances prior to their initial use. Follow the procedure for a monthly quality control check, refer to 7.4.1.

7.4. Perform a quality control check monthly on all balances.

7.4.1. Monthly Quality Control Check

7.4.1.1. Ensure that the balance is clean, conventional window-cleaning fluid may be used, if necessary.
7.4.1.2. Ensure that the balance is level. Adjust the leveling screws until the air bubble is within the inner circle of the level indicator. Press the internal adjustment key and allow the balance to complete the internal adjustment function.

7.4.1.3. Zero the empty balance and allow the reading to stabilize, i.e., the round circle in the upper left of the display has faded. Ensure that the reading is zero. Repeat if necessary.

7.4.1.4. For each standard weight – 200 g, 2 g and 0.1 g, place the standard weight in the center of the balance and allow the reading to stabilize, i.e., the round circle in the upper left of the display has faded, and record the weight on the Balance QCC form. The acceptable range for each weight is listed below.

Reference Standard Weight	Acceptable Range
200 g	199.998 - 200.002 g
2 g	1.998 - 2.002 g
0.1 g	0.098 g - 0.102 g

7.4.1.4.1. If the results are outside the acceptable range, correct any apparent problem, e.g., clean, level, and tare. If a problem was observed and corrected, record the activity and repeat the QCC. If no problem is observed or the result remains outside of the acceptable range, label the balance and the QCC form "out of service" and notify the DWI Blood Chemistry Technical Leader. A successful monthly QCC must be performed prior to placing the balance "back in service."

7.5. Balance operation

7.5.1. Leave the balance powered on. Prior to each use ensure that the monthly QCC, if applicable, has been successfully performed, refer to 7.4.1

7.5.2. Ensure that the balance is clean, conventional window-cleaning fluid may be used, if necessary.

7.5.3. Ensure that the balance is level. Adjust the leveling screws until the air bubble is within the inner circle of the level indicator. Press the internal adjustment key and allow the balance to complete the internal adjustment function.

7.5.4. Place the weighing vessel in the center of the balance and allow the reading to stabilize, i.e., the round circle in the upper left of the display has faded.

7.5.5. Zero the balance and ensure that the reading is zero. Repeat if necessary.

7.5.6. Place the weighing vessel on the balance, tare the balance and allow the reading to stabilize. Add the material to be weighed to the weighing vessel and allow the reading to stabilize.

7.6. Record maintenance and service, other than cleaning, leveling and taring, in a log maintained near the balance.

7.7. Maintain manufacturer's certificates, performance verification documentation, quality control check documentation and calibration certificates in the DWI Blood Chemistry Unit.

8. Procedure for Class A Volumetric Glassware

8.1. Volumetric glassware used in Blood Alcohol and Cannabinoid analyses and used to prepare internal standard solutions shall be Class A.

8.2. Inspect the glassware upon each use for damage and cleanliness.

8.2.1. Discard glassware that is damaged, e.g., chipped or cracked.

8.2.2. Discard glassware that cannot be thoroughly cleaned, e.g. discard pipets that do not drain cleanly, discard flasks that cannot be cleaned.

8.3. Maintain certificates and/or manufacturer specifications for volumetric glassware in the DWI Blood Chemistry Unit.

9. Procedure for Dispensers

9.1. Dispensers used to deliver volumes of solvents or prepared reagents shall have a manufacturer's specification of accuracy ± 1% and of reproducibility ± 0.1% and be appropriate for the intended use.

9.2. Prior to each use inspect the dispenser for ease of movement.

9.2.1. If the plunger and / or valve do not move freely, remove the glass bottle and immerse the assembly in hot water. After a few minutes and while the assembly is still immersed, slowly move the plunger. When the plunger and / or valve are moving freely in the barrel assembly fully dispense 6 to 10 cycles of the hot water. Remove the assembly from the water and reassemble the bottle and tubing. Record the activity in a log maintained in the DWI Blood Chemistry Unit.

9.2.2. If any other dispenser problem is observed, remove the dispenser from service, label it "out of service," and notify the DWI Blood Chemistry Technical Leader for replacement.

9.3. Dispensers shall be dedicated to and identified by the solvent or prepared reagent they contain.

9.4. Maintain manufacturer's specifications in the DWI Blood Chemistry Unit.

10. Procedure for Laboratory Environmental Monitor

10.1. The building environmental controls generally provide adequate laboratory environmental conditions for analysis. When environmental conditions are outside of acceptable levels they are apparent to a Blood Drug Chemist based on their sensory perception of the environment. When a Blood Drug Chemist perceives an abnormally high or low temperature or abnormally high humidity level the Blood Drug Chemist shall:

- Notify General Services Administration that there is an environmental control problem and provide the applicable room number
- Notify the DWI Blood Chemistry Technical Leader and Crime Laboratory Quality Manager of the environmental control issue
- Measure the laboratory environmental conditions using a hygrometer and thermometer. ~~Laboratory environmental conditions shall be monitored by a Blood Drug Chemist each working day using a hygrometer, thermometer and barometer.~~

10.2. Record the humidity, temperature and air pressure on the environmental logs.

10.3. If the humidity exceeds 80% or the temperature is not in the range of 15.5 – 32.2 °C, stop all analyses until environmental conditions return to acceptable levels.

10.3.1. All instruments shall be subjected to any post shutdown checks as described in the DWI Blood Chemistry Unit Technical Procedures.

11. Millipore Elix Advantage Water Purification System with E-POD Unit

11.1. Prior to dispensing deionized water ensure that the resistivity displayed on the E-POD unit is greater than 1.0 MOhm.cm.

11.2. To dispense water press down on the E-POD Unit plunger. Push slightly for low flow. Push down and hold for high flow. Push completely down for continuous high flow and push down again to stop.

11.3. Maintenance

11.3.1. Replace the Progard Pack, Reservoir Vent Filter and UV 254 lamp when prompted by the System. Refer to 14.9.

11.3.2. Clean the Inlet Strainer and RO Cartridge when prompted by the System. Refer to 14.9.

11.3.3. Record maintenance in a logbook maintained in the DWI Blood Chemistry Unit.

11.4. Record System alarms in the logbook and notify the DWI Blood Chemistry Unit Technical Leader if service is required.

12. Safety – Refer to the CCBI Crime Laboratory Safety Manual

13. Records

13.1. Balance log
13.2. Balance quality control check form
13.3. Refrigerator temperature log

13.4. Refrigerator maintenance log
13.5. Freezer temperature log
13.6. Freezer maintenance log
13.7. Environmental log
13.8. Pipette quality control check form
13.9. Pipette log
13.10. Dispenser log

14. References

14.1. Eppendorf Reference Operating Manual, 2001, Eppendorf AG, Hamburg.

14.2. Eppendorf Standard Operating Procedure for Pipettes, 2101, Eppendorf AG, Hamburg.

14.3. Nichiryo Accupenser Junior Dispenser Manual, 2009, Nichiryo, Tokyo.

14.4. Instructions for Installation, Use & General Maintenance of Your Glass Door Merchandiser MAN-004-R, 2009, Fogel.

14.5. T° Sentry Model 140 Digital Alarm Module Operating Instructions, 2004, Hampshire Controls Corporation.

14.6. Kenmore Elite Refrigerator Use & Care Guide, 2009, Sears, Roebuck and Co. Hoffman Estates, IL.

14.7. Thermo Scientific General Purpose, Flammable Storage, and Explosion Proof Laboratory Refrigerators and Freezers Installation and Operation Manual, 2010, Thermo Fisher Scientific.

14.8. Operating Instructions for Excellence Precision Balances XS Models – Part 1, 05/2012, Mettler-Toledo, Greifensee, Switzerland.

14.9. *Elix Advantage 3/5/10/15 System User Manual*, Millipore Corp, France, FTPF11337-V1.0, 02/2010.

14.10. Operating Instructions for Excellence Balances XS Models – Part 2, 10/2010, Mettler-Toledo, Greifensee, Switzerland.

14.11. Drug Chemistry Unit Technical Procedure for Balances.

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
3/27/13	2	Incorporated new balances.
1/16/15	3	Minor wording changes. Update record storage requirements and refrigerator temperature range. Remove reference to Millipore POD Pak.
11/06/2015	4	Update 10.1.

4: Technical Procedure for pH Meter

1. **Purpose / Scope** - This procedure provides direction for the use of the pH meter in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.2. Performance verification – The initial confirmation of the reliability of a previously or externally validated method or instrument.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. QCC – Quality control check

4. **Equipment, Materials, Reagents**

   4.1. Equipment

      4.1.1.pH Meter, Fisher Scientific Accumet Basic AB15/15+, with double-junction combination pH electrode and ATC probe

   4.2. Materials

      4.2.1. Deionized water
      4.2.2. Beaker
      4.2.3. Wash bottle

   4.3. Reagents

      4.3.1.pH buffers, certified by manufacturer: 4.0, 7.0 and 10.0
      4.3.2. Electrode storage solution, potassium hydrogen phthalate-potassium chloride
      4.3.3. Electrode fill solution, saturated potassium chloride

5. **Standardization of pH Meter**

   5.1. Standardize the pH meter daily, when in use, with two buffer solutions with values that bracket the desired measuring range, e.g., pH 4.0 and pH 7.0.

   5.2. If necessary, fill the electrode with the electrode fill solution.

5.3. Ensure that the filling hole of the electrode is open.

5.4. Press and release the **mode** key until the digital display indicates pH Mode.

5.5. Press the **setup** key twice and then press **enter** key to clear an existing standardization.

5.6. Immerse the rinsed electrode and temperature probe into a beaker containing a buffer solution and stir moderately.

5.7. Press the **std** key to access the Standardize mode. A selected buffer group is displayed briefly.

5.8. Wait for reading to stabilize.

5.9. Press the **std** key again to initiate standardization. The meter will automatically recognize the buffer and then the meter returns to the measure screen.

5.10. Repeat 5.6 -5.8 with a second buffer solution. When the meter accepts the second buffer, it will briefly display the percent slope associated with the electrode's performance prior to returning to the measure mode. If the electrode is within the range of 90% - 102%, the "good electrode" message will appear. If the electrode is outside this range, the meter will display the "electrode error" message and will not return to the measure screen until the user presses the **enter** key.

5.10.1. If the electrode is outside of the "good electrode" range of 90% - 102% an "electrode error" message will appear. Rinse and fill the electrode with electrode filling solution and repeat the standardization.

5.10.1.1. If the problem persists test the meter for correct operation.

5.10.1.2. Install the BNC (input) shorting cap.

5.10.1.3. Press **mode** to access the mV mode and record the mV reading. If the meter reads 0 ± 1 mV the meter is measuring correctly, proceed to 5.10.1.4. If the reading is outside the acceptable range, mark the pH meter log "out of service" and notify the DWI Blood Chemistry Technical Leader for scheduling of repair. Record the activity and result in the pH meter log.

5.10.1.4. If the pH meter is functioning correctly replace the electrode, fill it with electrode filling solution, soak it for 2 – 4 hours in electrode storage solution and perform the standardization with the new electrode. Record the activity in the pH meter log.

5.11. Record date, initials, buffers and lot numbers, slope and message displayed in the pH meter log.

5.12. Remove the electrode and temperature probe from the buffer solution and rinse with distilled water.

6. Procedure

6.1. Prior to use daily standardize the pH meter, refer to section 5.

6.2. Ensure that the filling hole of the electrode is open.

6.3. Set the **mode** key to pH measure.

6.4. Rinse the electrode and temperature probe with deionized water and blot dry.

6.5. Immerse the electrode and temperature probe into a beaker containing the solution and stir moderately.

6.6. Wait for reading to stabilize and then record the reading.

6.7. Remove the electrode and thermometer from the solution.

6.8. Rinse the electrode and thermometer with deionized water before and blot dry.

6.9. Immerse the electrode in the electrode storage solution when the pH meter is not in use.

6.10. Record the date, initials, measured liquid identity and observed pH in the pH meter log.

6.11. Maintain the pH meter log and buffer certificates in a pH meter logbook in the DWI Blood Chemistry Unit near the pH meter.

7. Safety

7.1. Refer to the CCBI Crime Laboratory Safety Manual

8. Records

8.1. pH meter log

9. References

9.1. Accumet Basic AB15 User Manual, August 2006, Fisher Scientific.

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated Section 3, corrected numbering.

5: Technical Procedure for Use of the Hamilton Microlab 625 Diluter to Prepare Samples for Blood Alcohol Determination Using Headspace Gas Chromatography

1. **Purpose / Scope** - This procedure provides direction for the initial setup, maintenance, performance checks and usage of the Hamilton Diluter ~~infrared spectrometers Spectrometer~~ in the DWI Blood ~~Drug~~ Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification.

2. **Definitions**

   2.1. Performance verification - The initial confirmation of the reliability of a previously or externally validated method or instrument.
   2.2. Quality control check (QCC) - Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.3. Reference Material – Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. BAC – Blood alcohol content
   3.3. QCC – Quality Control Check

4. **Equipment, Materials, and Reagents**

   4.1. Equipment

      4.1.1. Hamilton Microlab 625 Dilutor with 250 µl and 2500 µl and Hamilton BFP Syringes located in a Biological Safety Cabinet

   4.2. Materials

      4.2.1. Headspace sample vials with caps
      4.2.2. Liquid waste container, e.g., 250 ml beaker
      4.2.3. Lint free wipes
      4.2.4. Reservoir for the internal standard solution
      4.2.5. Deionized water

   4.3. Reagents

4.3.1. BAC Internal Standard Solution, refer to the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography

5. Performance Verification

5.1. Upon initial setup perform the Hamilton Microlab 600 Qualification Procedure, refer to11.3.

5.2. Complete a successful daily QCC as described in the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography.

5.3. Maintain the performance verification results in the DWI Blood Chemistry Unit.

6. Maintenance

6.1. Record any maintenance in the Hamilton Dilutor Log.

6.1.1. Replace the syringes annually at the time of annual calibration.

6.2. A successful quality control check must be performed prior to analyzing samples, refer to 8.

7. Calibration

7.1. The instrument shall be calibrated annually after the first year of service by an approved vendor.

7.2. Record calibrations in the Hamilton Dilutor Log and maintain calibration certificates in the DWI Blood Chemistry Unit.

8. Quality Control

8.1. Refer to the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography for quality control check procedures.

9. Procedure

9.1. Cover or seal liquids containing volatile substances to prevent evaporation of the volatiles. Fill a reservoir with the BAC internal standard solution and place the left diluent tube in the internal standard solution. Do not seal or cover the reservoir tightly - a vacuum may form and interfere with functioning.

9.2. Turn power on with the switch on the front of the unit.

9.3. Selections on the display screen may be entered by pressing the icon on the screen. Values may be entered via the numeric keypad that appears on the screen. Arrow keys on the screen may be used to move from one data entry field to another.

9.4. Method programming. The BAC Method is initially programmed as follows and may be adjusted to accommodate instrument performance. Document any adjustments in the Hamilton Dilutor log and perform a successful calibration and daily QCC according to the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography prior to analyzing samples. Entries to be selected are in bold face type. It is not necessary to program the method for each use, proceed to 9.5.

9.4.1. Configuration – Syringe Set-up			
9.4.1.1.	**Syringe Size**	**Left – 2500**	**Right – 250**
9.4.1.2.	**Syringe Flow Rate**	**Left – 625**	**Right – 125**
9.4.1.3.	**Initialize Flow Rate**	**Left – 625**	**Right – 125**
9.4.1.4.	**Return Steps**	**Left – 24**	**Right – 24**
9.4.1.5.	**Back-Off Steps**	**Left – 96**	**Right – 80**
9.4.2. Configuration - Valve Type		**Dual Diluter**	
9.4.3. Configuration – Trigger		**Either**	
9.4.4. Wizards – Dilution			
9.4.4.1.	**Left Syringe**	**1800**	
9.4.4.2.	**Right Syringe**	**200**	
9.4.4.3.	**Air Gap**	**15**	
9.4.4.4.	**Refill**	**On**	
9.4.4.5.	**Final Volume**	**2000**	
9.4.5. Wizards – Advanced			
9.4.5.1.	**Air Gap Mode**	**Auto**	
9.4.5.2.	**Air Gap Delay**	**1.5 S**	
9.4.5.3.	**Wash Mode**	**Off**	
9.4.5.4.	**Program Mode**	**Diluent and Sample**	
9.4.5.5.	**Fill Speed**	**Left – 625**	**Right – 100**
9.4.5.6.	**Aspirate Speed**	**Left – 312**	**Right – 100**
9.4.5.7.	**Dispense Speed**	**Left – 625**	**Right – 100**
9.4.5.8.	**Probe Light Green**	**Trigger**	
9.4.5.9.	**Probe Light Red**	**Never**	

9.5. Using the BAC Method

9.5.1. Ensure the left diluent tube is in the internal standard solution.
9.5.2. Prime the system by pushing the **Prime** button on the front of the instrument.
9.5.3. Prime the system several times and ensure that the air has been purged from the tubes.
9.5.4. Select the **Favorites** icon, then the **Dilution** icon and then select **BAC**.
9.5.5. The following steps can be activated by pressing the **Run/Pause** key or by pressing the trigger button on the hand probe.

9.5.6. Collect an aliquot of the sample to be analyzed by placing the tubing on the hand probe into the sample and aspirating the sample by pressing the trigger button. The sample is contained in the tubing and should never be aspirated into the syringe. An air gap separates the sample in the tubing from the internal standard solution, preventing the two from mixing.

9.5.7. When the submitted sample size allows, discard this aliquot by dispensing to a waste container.

9.5.8. Wipe the outside of the tubing with a clean lint free wipe to remove any residue.

9.5.9. Collect an aliquot of the sample to be analyzed by placing the tubing on the hand probe into the sample and aspirating the sample be pressing the trigger button.

9.5.10. Wipe the outside of the tubing with a clean lint free wipe to remove sample residue.

9.5.11. Dispense the sample aliquot and diluent into a headspace vial, labeled to correspond to the sample, by pressing the trigger button. The sample is dispensed first, followed by the internal standard solution, which flushes the sample from the tubing. After the sample and diluent are dispensed into the head space vial, the left syringe automatically refills with the internal standard solution and an air gap is created in the tubing.

9.5.12. Seal the vials by screwing the top onto the headspace vial.

9.5.13. Wash the tubing between each sample by aspirating a minimum of one deionized water sample and then dispensing the water/internal standard solution into a waste container. ~~This washing procedure flushes the tubing with water and 1.8 ml of diluent.~~

9.5.14. Flush the tubing with a mixture of approximately 10% Clorox and deionized water or equivalent daily, after use, to remove protein build-up and prevent bacterial growth in the tubing.

10. Safety

10.1. Refer to the CCBI Crime Laboratory Safety Manual.

11. References

11.1. *Hamilton Microlab 600 Series Basic Manual*, April 2010, Hamilton Company, Nevada, USA.

11.2. *Hamilton Microlab 600 Series Basic Manual*, March 2011, Hamilton Company, Nevada, USA.

11.3. *Hamilton Microlab 600 Qualification Procedure*, Hamilton Company, Nevada, USA.

12. Records

12.1. Hamilton Dilutor Log

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3.
1/16/15	2	Correct reference in section 1 and included reference to maintenance. Include requirement for annual syringe replacement. Include instruction for aspirating and discarding an initial aliquot when sample size allows. Require a minimum of one rinse with deionized water between samples.

6: Technical Procedure for Biotage TurboVap LV Evaporator

1. **Purpose / Scope -** This procedure provides direction for the use of the Biotage TurboVap LV Evaporator to concentrate extracted samples in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification.

2. **Definitions**

   2.1. N/A

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis

4. **Equipment and Materials**

   4.1. Equipment

      4.1.1. Biotage TurboVap LV Evaporator located in a fume hood with a 110/120 VAC power supply

   4.2. Materials

      4.2.1. Nitrogen, ultra high purity grade
      4.2.2. Deionized water
      4.2.3. 16 x 125mm or 13 x 100mm glass test tubes
      4.2.4. 16 x 125mm or 13 x 100mm TurboVap LV test tube rack
      4.2.5. Clear bath or equivalent antibacterial agent

5. **Procedure**

   5.1. Record any maintenance or comments in the TurboVap Log. The addition of water and antibacterial agent need not be recorded.

   5.2. Fill water bath, if necessary, with deionized water and antibacterial agent.

   5.3. Place the appropriately sized test tube rack into the water bath.

   5.4. Power on the TurboVap with the switch at lower left-hand side of instrument.

   5.5. Set water bath temperature with the TEMP numeric push-wheel and allow the water to reach the set temperature. Water temperature is stable when the TEMP light stops blinking.

5.6. Place the test tubes containing extracted samples, up to 50, into the rack in the water bath.

5.7. Set the TIME push-wheel to the desired drying time, e.g., 10 ml of methylene chloride, at 35 degrees C, at 12 psi of nitrogen, will evaporate in approximately 20 minutes.

5.8. Select the row(s) containing the extracted sample(s) by pressing its corresponding TUBE STATIONS push-button, choose the button that aligns horizontally with the row.

5.9. Turn on the nitrogen and set the gas pressure at 10-12 psi with the regulator adjustment valve located on the left side of the instrument.

5.10. Check the fume hood display and ensure that no error messages are displayed. If there is an error do not use the evaporator and notify the DWI Blood ~~Drug~~ Chemistry Technical Leader for scheduling of fume hood service.

5.11. Press the START button.

5.12. Monitor the samples and remove the test tubes immediately upon evaporation of the solvent. Increase the time period setting on the instrument if needed. The TurboVap will sound an alarm when the set time period has expired.

6. Safety

6.1. Refer to the CCBI Crime Laboratory Safety Manual

7. Records

7.1. TurboVap log

8. References

8.1. TurboVap LV Evaporator Users Manual, 2010, Biotage Corporation, Charlotte, NC.

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3.
1/16.15	2	Corrected reference to technical leader in 5.10

7: Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography

1. **Purpose / Scope** - This procedure specifies the required elements for calibration and use of the headspace gas chromatograph to determine alcohol (ethanol, methanol, and isopropanol) and acetone concentration in blood in the DWI Blood Chemistry Unit of the CCBI Crime Laboratory.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.2. Performance verification – The initial confirmation of the reliability of a previously or externally validated method or instrument.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. BAC – Blood alcohol concentration
   3.3. GC – Gas chromatograph
   3.4. QCC – Quality control check

4. **Equipment, Materials and Reagents**

   4.1. Equipment and Materials

   4.1.1. Agilent 7890 Gas chromatograph equipped with flame ionization detectors with Agilent or equivalent DB-ALC1 (front) 30m x 0.535mm x 3.00µm and DB-ALC2 (back) 30m x 0.535mm x 2.00µm capillary columns, G6509 headspace autosampler, data station and printer.
   4.1.2. Hamilton-Microlab 625 dilutor
   4.1.3. Mettler XS1003S Balance
   4.1.4. Volumetric flasks, Class A: 100, 1000, and 2000 mL, to contain
   4.1.5. Volumetric pipettes, Class A: 1, 4, 5, 8, 10, 20, and 50 mL, to deliver ~~contain~~

   4.2. Materials

   4.2.1. Headspace vials with sealing caps
   4.2.2. Deionized water
   4.2.3. Helium gas, UHP Grade
   4.2.4. Hydrogen gas, UHP Grade
   4.2.5. Nitrogen gas, UHP Grade

4.2.6. Compressed Air, Zero Grade

4.3. Reference Materials

4.3.1. Ethanol, 200 proof, USP or ACS Grade
4.3.2. n-Propanol (1-propanol), ACS Grade
4.3.3. Isopropanol (2-propanol), ACS Grade
4.3.4. Acetone, ACS Grade
4.3.5. Methanol, ACS Grade
4.3.6. Multi-component solutions, NIST traceable, containing ethanol, methanol, acetone and isopropanol. Concentrations - 0.010 g / 100 mL, 0.050 g/100 mL, 0.100 g/100 mL and 0.400 g/100 mL.

4.4. Prepared Reagents

4.4.1. BAC Stock Calibration Solution

4.4.1.1. Prepare a solution containing 1.000 gram / 100 mL each of ethanol, methanol, acetone, and isopropanol reference material. Record the weight of each component.

Example: Weigh 10.00 g each of ethanol, methanol, acetone, and isopropanol into a beaker. Quantitatively transfer each into a single 1000 mL volumetric flask. Bring the flask to volume with deionized water**.**

4.4.1.2. Lot Number: Eight digit format year/month/day/BacStockCal/initials of preparer.

Example: 20130101BacStockCalXXX

4.4.1.3. Expiration: One year.

4.4.1.4. Storage: Refrigerate.

4.4.1.5. QCC: Not applicable, see BAC working calibration solutions

4.4.2. BAC Working Calibration Solutions

4.4.2.1. Prepare 0.010, 0.040, 0.080, 0.200, and 0.500 gram / 100 mL BAC working calibration solutions from the BAC stock calibration solution using the appropriate Class A pipette for each solution.

Example: Pipet 1, 4, 8, 20, and 50 mL of the stock calibration solution into separate 100 mL volumetric flasks containing approximately 50 mL deionized water. Bring each flask to volume with deionized water**.**

4.4.2.2. Lot Numbers: Eight digit format year/month/day, Bac, three decimal place concentration, Cal, initials of preparer.

Example: 20130101Bac0.010CalXXX

4.4.2.3. Expiration: Three months.

4.4.2.4. Storage: Refrigerate.

4.4.2.5. QCC: Successful calibration, refer to 8.

~~**4.4.3.BAC Stock Verification Solution**~~

~~**4.4.3.1.** Prepare a solution containing 1.000 gram / 100 mL each of ethanol, methanol, acetone, and isopropanol reference material. Record the weight of each component.~~

~~**4.4.3.2.** Example: Weigh 10.00 g each of ethanol, methanol, acetone, and isopropanol into a beaker. Quantitatively transfer each into a single 1000 mL volumetric flask. Bring the flask to volume with deionized water~~

~~**4.4.3.3.** Lot Number: Eight digit format year/month/day/BacStockVer/initials of preparer.~~

~~Example: 20130101BacStockVerXXX~~

~~**4.4.3.4.** Expiration: One year.~~

~~**4.4.3.5.** Storage: Refrigerate.~~

~~**4.4.3.6.** QCC: Not applicable, see BAC working verification solutions.~~

~~**4.4.4.BAC Working Verification Solutions**~~

~~**4.4.4.1.** Prepare 0.010, 0.040, 0.080, 0.100, 0.200, or 0.500 gram/100 mL verification solutions from the BAC stock verification solution using the appropriate Class A pipette for each solution.~~

~~Example: Pipet 1, 4, 8, 10, 20, and 50 mL of the stock verification solution into separate 100 mL volumetric flasks containing approximately 50 mL deionized water. Bring each flask to volume with deionized water.~~

~~**4.4.4.2.** Lot Numbers: Eight digit format year/month/day, Bac, three decimal place concentration, Ver, initials of preparer.~~

~~**4.4.4.2.1.** Example: 20130101Bac0.010VerXXX~~

~~**4.4.4.3.** Expiration: Three months.~~

~~**4.4.4.4.** Storage: Refrigerate.~~

~~**4.4.4.5.** QCC: Meet the requirements detailed in 8.8.3.1. thru 8.8.3.3.~~

4.4.5.BAC Internal Standard Solution (n-propanol)

4.4.5.1. Prepare a 0.050 gram / 100 mL solution of n-propanol reference material.

Example: weigh 1.00 gram of n-propanol into a beaker. Quantitatively transfer to a 2000 mL volumetric flask. Bring the flask to volume with deionized water.

4.4.5.2. Lot Number: Eight digit format year/month/day/BacIS/initials of preparer.

Example: 20130101BacISXXX

4.4.5.3. Expiration: Three months.

4.4.5.4. Storage: room temperature.

4.4.5.5. QCC: Successful calibration, refer 8.

5. Gas Chromatograph Performance Verification for New Instruments

5.1. New gas chromatographs shall be installed by a manufacturer representative and shown to meet manufacturer requirements.

5.2. Complete a performance verification on new gas chromatographs prior to use for casework. Maintain the data in the DWI Blood Chemistry Unit.

5.3. Include in the performance verification:

5.3.1. Successful calibration, refer to 8.

5.3.2. Successful daily QCC, refer to 9.1.1.

5.3.3. Analysis of a minimum of five preparations of multi-component reference material from 4.4.1. at concentrations of 0.010, 0.050, 0.100 and 0.400 g / 100 mL, over at least a two day period. All quality control requirements must be met.

5.3.3.1. The % RSD at each concentration must be 2% (5% for acetone) or less.

5.3.3.2. The individual concentration results must be within ± 5% (10% for acetone) of the target concentration for concentrations of 0.040 g / 100 mL or greater. For samples with target concentrations of less than 0.040 g / 100 mL, the individual concentration results must not differ from the target concentrations by more than ± 0.002 g / 100 mL.

5.3.4. The analysis of three preparations of reference material at 0.004 g / 100 mL.

5.3.4.1. All components must be identified.

5.3.5. The analysis of five preparations of 0.080 g / 100 mL multi-component samples prepared using negative blood (containing sodium fluoride preservative and oxalate anticoagulant) and five preparations of negative blood.

5.3.5.1. The % RSD at each concentration must be 2% (5% for acetone) or less.

5.3.5.2. The individual concentration results must be within ± 5% (10% for acetone) of the target concentration.

6. Gas Chromatograph Maintenance

6.1. Record all maintenance in the maintenance log at the time it is performed.

6.2. When maintenance is performed, the instrument shall be out of service until the required daily system check and/or calibration is successfully completed and recorded in the instrument log. File any generated data in the instrument logbook maintained near the instrument.

6.3. Suggested Routine Maintenance Schedule

6.3.1. This is a suggested maintenance schedule. Instrument use may alter the need for maintenance. The maintenance schedule shall be determined by the DWI Blood Chemistry Unit Technical Leader based upon instrument usage.

6.3.2. Septum

6.3.2.1. Replace weekly when in use.

6.3.2.2. A successful daily QCC, refer to 9.1.1, shall be performed prior to analyzing samples.

6.3.3. Syringe

6.3.3.1. Replace every six months.

6.3.3.2. A successful daily QCC, refer to 9.1.1, shall be performed prior to analyzing samples.

6.3.4. Liner

6.3.4.1. Replace every twelve months.

6.3.4.2. A successful daily QCC, refer to 9.1.1, shall be performed prior to analyzing samples.

6.3.5. Jet

6.3.5.1. Inspect every twelve months, replace as needed.

6.3.5.2. A successful daily QCC, refer to 9.1.1, shall be performed prior to analyzing samples.

6.3.6. Column

6.3.6.1. Replace every twelve months.

6.3.6.2. A successful calibration, refer to 8, and daily QCC, refer to 9.1.1, shall be performed prior to analyzing samples.

6.3.7. Non-routine Maintenance

6.3.7.1. All non-routine maintenance shall, at a minimum, be followed by a successful daily QCC, refer to 9.1.1, prior to analyzing samples.

6.3.7.2. Non-routine maintenance shall be evaluated by the DWI Blood Chemistry Unit Technical Leader to determine the need for recalibration prior to analyzing samples.

6.3.8. Shutdown

6.3.8.1. Successful daily QCC, refer to 9.1.1, shall be performed following any GC or autosampler shutdown.

6.3.8.2. Record the shutdown in the maintenance log.

7. Gas Chromatograph Parameters

7.1. The stated method parameters are initial settings that may be adjusted to accommodate instrument performance. Document any adjustments in the instrument log and perform a successful calibration, refer to 8, and daily QCC, refer to 9.1.1, prior to analyzing samples.

7.2. Gas Chromatograph Method Parameters

7.2.1. Isothermal Column Temperature: 35 °C
7.2.2. Injector Temperature: 200 °C
7.2.3. Detector Temperature: 200 °C
7.2.4. Column Flow: 9 mL/min, nominal, constant flow
7.2.5. Split ratio: 2:1
7.2.6. Run Time: 5:00 minutes
7.2.7. Detector make up flow: 18 mL/min
7.2.8. Detector H2 flow: 30 mL/min
7.2.9. Detector Air Flow: 300 mL/min

7.3. Headspace Autosampler Method Parameters

7.3.1. Incubation Temperature: 70 °C
7.3.2. Incubation Time: 480 seconds
7.3.3. Agitator Speed: 250 rpm
7.3.4. Agitator On/Off: 5 seconds on / 30 seconds off
7.3.5. Runtime 5:00 minutes
7.3.6. Syringe temperature 75 °C
7.3.7. Sample volume 1000 µL
7.3.8. Syringe fill speed: 100 µL / second
7.3.9. Pull-up delay: 2 seconds
7.3.10. Injection speed: 700 µL / second
7.3.11. Pre-inject delay / Post-inject delay: 0.5 second
7.3.12. Flush time: 120 seconds

7.4. Data Station Method Parameters

7.4.1. The peak retention times may ~~shall~~ be updated daily, when in use, to the retention times of the last injection of the 0.100 g/100 mL NIST traceable multi-component solution in the current Daily QCC.

7.4.2. The peak retention time windows shall be set to the peak retention time ± 0.050 minutes + 0.5 % of the peak retention time.

8. BAC Calibration

8.1. Calibrate the GC upon preparation of a new lot of Internal Standard solution and after instrument maintenance that may affect the calibration.

8.2. Prepare each of the calibration solutions from 4.4.2 in duplicate according to 9.2.

8.3. Chromatograph the calibration samples on the gas chromatograph

8.4. Update the calibration with the calibration samples.

8.4.1. The response at each concentration shall be determined by the average response of the duplicates analyzed at that concentration.

8.4.2. The retention time for each component shall be that of the final calibration sample injected.

8.4.3. The calibration curve shall be fitted to a linear model with equal weighting and with the origin included.

8.4.4. The calibration curves for each component shall show a coefficient of determination, r^2, of 0.995 or greater. If the calibration has a coefficient of determination of less than 0.995, take appropriate action, e.g., maintenance and / or preparation of new solution(s), and repeat the calibration. Record all calibrations and maintenance in the instrument logbook.

8.5. Save the data analysis method according to the format "BacCal" and eight digit format year/month/day.

Example: BacCal20130101

8.6. Reprocess the calibration samples with the updated method.

8.6.1. Each component must be identified by the instrument software on both columns and be visually baseline resolved.

8.6.2. For those with target concentrations of 0.040 g / 100 mL or greater, each component's individual quantitation results must be within +/- 5.0 % of the target.

8.6.3. For those with target concentrations of less than 0.040 g / 100 mL, each component's individual quantitation results must be within +/- 0.002 g/100 mL of the target concentration.

8.7. If the calibration is unacceptable for ethanol, take appropriate action, e.g., maintenance or new solution preparation, and repeat the calibration. If the calibration is unacceptable for a component other than ethanol either take appropriate action, e.g., maintenance or new solution

preparation, and repeat the calibration or record the unacceptable component on the calibration data and in the calibration log. Do not perform quantitations of the unacceptable component.

8.8. BAC Calibration Verification

8.8.1. Prepare each of the NIST traceable multi-component solutions (0.010, 0.050, 0.100 and 0.400 g / 100 mL) in duplicate according to 9.2.

8.8.2. Chromatograph the verification samples on the gas chromatograph.

8.8.3. Quantitate the verification samples with the updated calibration.

8.8.3.1. Each component must be identified by the instrument software on both columns and be visually baseline resolved.

8.8.3.2. For the duplicates with target concentrations greater than 0.04 g/100 mL, evaluate each component's quantitation results according to the equation

$$\%\text{diff} = \frac{100 \times [(\text{highest measured concentration}) - (\text{lowest measured concentration})]}{(\text{lowest measured concentration})}$$

The percent difference must be ≤ ~~within ±~~ 5.0 % (10 % for acetone). Any components that do not meet this requirement will not be quantitated.

8.8.3.2.1. Each component's individual quantitation results must be within ± 5.0 % (10 % for acetone) of the target concentration. Any components that do not meet this requirement shall not be quantitated.

8.8.3.3. For the 0.01 g / 100 mL duplicates, each component's individual quantitation results must be within +/- 0.002 g / 100 mL of the target concentration. Any components that do not meet this requirement shall not be quantitated.

8.8.4. If the calibration is unacceptable for ethanol, take appropriate action, e.g., maintenance or new solution preparation, and repeat the calibration. If the calibration is unacceptable for a component other than ethanol either take appropriate action, e.g., maintenance or new solution preparation, and repeat the calibration or record the unacceptable component on the calibration data and in the calibration log. Do not perform quantitations of the unacceptable component.

8.9. Prepare a file folder labeled with the name of the calibrated method, refer to 8.5. Maintain this file in the DWI Blood Chemistry Unit. This Calibration file shall include:

8.9.1. Reprocessed calibration sample instrument printouts
8.9.2. Verification sample instrument printouts

8.9.3. Lot numbers of all prepared reagents: calibration solutions and internal standard solution
8.9.4. Lot numbers of the NIST traceable multi-component solutions
8.9.5. Calibration table and the r^2 values of each component.

8.10. Record each calibration in the calibration log with the date, lot number of internal standard used, internal standard expiration date and operator initials.

9. Procedure

9.1. Quality Control Checks

9.1.1. Daily Quality Control Check

9.1.1.1. The daily quality control check must be performed in the twenty-four hours preceding any sample analysis. The daily quality control check is valid for twenty four hours after the injection of the first verification standard.

9.1.1.2. Prepare each of the NIST traceable multi-component solutions, 0.010, 0.050, 0.100 and 0.400 g/100mL, in duplicate according to 9.2. Prepare a water blank in duplicate using deionized water according to 9.2.

9.1.1.3. Chromatograph the prepared samples in a single sequence with ~~the~~ a single water blank at the beginning followed by the NIST traceable duplicates from low to high concentration and a single water blank after the 0.400 g/100 mL NIST traceable multi-component solution samples.

9.1.1.4. Quantitate the samples with the current data analysis method corresponding to the internal standard used.

9.1.1.4.1. Each NIST traceable multi-component solution sample must meet the requirements stated in 8.8.3.1 through 8.8.3.3.

9.1.1.4.2. The water blank must not contain any identifiable methanol, ethanol, isopropanol, acetone or any other identifiable volatile.

9.1.1.5. If the QCC is unacceptable for ethanol, take appropriate action, e.g., maintenance or new solution preparation, and repeat the QCC. If the QCC is unacceptable for a component other than ethanol either take appropriate action, e.g., maintenance or new solution preparation, and repeat the QCC or record the unacceptable component on the QCC data and in the instrument log. Do not perform quantitations of the unacceptable component

9.1.1.6. Prepare a file folder labeled "BacQCC" followed by the date in yyyymmdd format. Maintain this file in the DWI Blood Chemistry Unit. This QCC file shall include:

9.1.1.6.1. NIST traceable multi-component solution sample instrument printouts
9.1.1.6.2. NIST traceable multi-component solution lot numbers
9.1.1.6.3. Water blank sample instrument printouts
9.1.1.6.4. Lot number and expiration of internal standard solution

9.1.1.7. Record each daily QCC in the instrument log with initials, date, time of the first injection, lot number of internal standard used, internal standard expiration date and operator initials.

9.1.2. In-Sequence Quality Control Checks

9.1.2.1. A sample sequence must contain a minimum of 10 % control samples.

9.1.2.2. The sequence must contain at least one positive control sample and one negative control sample, each prepared in duplicate.

9.1.2.3. The first and last samples of a sequence shall be control samples with any remaining required control samples evenly distributed throughout the batch. The samples and controls should be prepared in the same order in which they will be analyzed.

9.1.2.4. Select the target concentration of the positive control samples to avoid duplicate concentrations within a sequence when possible.

9.1.2.5. Negative control

9.1.2.5.1. Prepare a deionized water sample according to 9.2.

9.1.2.5.2. Negative controls must not contain any identifiable methanol, ethanol, isopropanol or acetone.

9.1.2.6. Positive control

9.1.2.6.1. Prepare the number and concentrations needed of the NIST traceable multi-component solutions, refer to 4.3.6~~., or BAC working verification solutions, refer to 4.4.4~~.

9.1.2.6.2. Each positive control sample component must meet the requirements in 8.8.3.1 through 8.8.3.3.

9.1.2.7. Record any in-sequence QCC sample components that do not meet all requirements in the instrument log and follow the Crime Laboratory Administrative Procedure for Corrective and Preventive Action.

9.1.2.7.1. If an in-sequence QCC sample is found to be unacceptable for ethanol, all cases in the sequence after the last acceptable positive quality control sample shall be reanalyzed. This shall be documented in the case file. Data generated after the last acceptable positive control sample shall not be used to identify or quantify ethanol in casework.

9.1.2.7.2. If an in-sequence QCC sample is found to be unacceptable for a component other than ethanol, cases in the sequence after the last acceptable positive quality control sample for that component shall be reanalyzed if that component is present. This shall be documented in the case file. Data generated after the last acceptable positive control sample shall not be used to identify or quantify that component in casework.

9.1.2.7.3. Record each sequence in the instrument log with initials, date and time of the first injection, internal standard solution lot number and expiration date.

9.1.2.8. Include the in-sequence QCC data in the "BacQCC" file from 9.1.1.6. The file shall include:

9.1.2.8.1. Sequence table
9.1.2.8.2. Positive and negative control instrument printouts
9.1.2.8.3. Lot numbers of the BAC working verification solutions and / or NIST traceable multi-component solutions

9.2. Alcohol and Acetone Concentration in blood

9.2.1. Sampling

9.2.1.1. Allow all solutions and samples to be analyzed to equilibrate to room temperature.

9.2.1.2. Ensure that blood samples are homogenous by shaking and/or vortexing.

9.2.1.2.1. If a homogenous sample cannot be obtained due to the presence of clots, a notation shall be made in the case file and the alcohol concentration shall be calculated according to 9.2.7.6.

9.2.1.2.2. If a homogenous sample cannot be obtained because the blood cells have been separated from the liquid, a notation shall be made in the case file and the alcohol concentration shall be calculated according to 9.2.7.6.

9.2.1.2.3. If a homogenous sample cannot be obtained for any other reason, a notation shall be made in the case file detailing the condition of the sample and its handling.

9.2.2. With the Hamilton Microlab 625 Dilutor, refer to the DWI Blood Chemistry Unit Technical Procedure for Use of the Hamilton Microlab 625 Dilutor to Prepare Samples for Blood Alcohol Determination, deliver 1.80 mL of the BAC Internal Standard Solution and 0.20 mL of the liquid to be analyzed into a headspace vial and cap securely.

9.2.3. Prepare each liquid to be analyzed in duplicate and chromatograph using the BAC method.

9.2.4. Quantitate each sample using the current calibrated BAC method corresponding to the lot of BAC Internal Standard Solution used in sample preparation.

9.2.5. The value of the multiplier in the sequence table shall be 10.

9.2.6. Identification of alcohol and acetone

9.2.6.1. Ethanol, methanol, isopropanol or acetone shall be integrated in the appropriate retention time window on both columns in both sample preparations to be identified.

9.2.6.2. Include the sample instrument prinouts, calibration file reference and quality control check file reference the case file.

9.2.6.3. Reporting

9.2.6.3.1. Refer to the DWI Blood Chemistry Unit Technical Procedure for DWI Blood Chemistry Analysis for reporting of identified alcohols and acetone.

9.2.7. Determination of alcohol or acetone concentration

9.2.7.1. The mean of the four measured values obtained for each component is the concentration of that component.

9.2.7.2. The Blood Alcohol Concentration is the sum of the means of the concentrations of the identified alcohols in a blood sample. Each mean shall be truncated to the hundredths place prior to summation.

9.2.7.3. The concentration of acetone shall be truncated to the hundredths place.

9.2.7.4. Reanalyze samples with identified alcohol or acetone values greater than or equal to 0.040 g / 100 mL that exhibit greater than 5 % (10 % for acetone) difference as described by the equation:

$$\%\text{diff} = \frac{100 \times [(\text{highest measured concentration}) - (\text{lowest measured concentration})]}{(\text{lowest measured concentration})}$$

9.2.7.5. Reanalyze samples with identified alcohol or acetone values less than 0.040 g / 100 mL that exhibit a difference between the highest and lowest measured concentrations greater than 0.004 g / 100 mL.

9.2.7.6. Clotted blood samples that cannot be rendered homogenous and samples in which the blood cells have been separated from the liquid (including serum and plasma) shall be converted to an equivalent whole blood alcohol concentration by dividing the alcohol concentration by 1.2 ~~1.18~~ to compensate for the whole blood:serum alcohol distribution ratio.

9.2.7.7. Reporting

9.2.7.7.1. Refer to the DWI Blood Chemistry Unit Technical Procedure for DWI Blood Chemistry Analysis for reporting of identified alcohols and acetone.

10. Calculations

10.1.1.1. Alcohol or acetone values percent difference:

$$\%\text{diff} = \frac{100 \times [(\text{highest measured concentration}) - (\text{lowest measured concentration})]}{(\text{lowest measured concentration})}$$

10.1.1.2. Conversion of non-homogenous samples and samples in which the blood cells have been separated from the liquid to an equivalent whole blood alcohol concentration: divide the alcohol concentration by 1.2 ~~1.18~~.

11. Uncertainty of Measurement

11.1. Refer to the DWI Blood Chemistry Unit Technical Procedure for Uncertainty of Measurement.

12. Limitations

12.1. Clotted samples that cannot be rendered homogenous and samples in which the blood cells have been separated from the liquid (including serum and plasma) shall be converted to an equivalent whole blood alcohol concentration by dividing the measured alcohol concentration by 1.2 ~~1.18~~ to compensate for the whole blood:serum (plasma) alcohol distribution ratio.

12.2. Refer to the Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography validation data in the DWI Blood Chemistry Unit for limit of detection and limit of quantitation.

12.3. Quantitation values greater than the highest calibration standard, 0.50 g / 100 ml, may be diluted and reanalyzed or reported as "greater than 0.50 gram per 100 ml."

13. Safety

13.1. Refer to the Crime Laboratory Safety Manual.

14. References

14.1. Randall C. Baselt. *Disposition of Toxic Drugs and Chemicals in Man.* 8th Ed. (2008): 561 – 565.

14.2. Agilent 7890A GC User Information, Agilent Instrument Utilities Version B.1.06.11343.1852.

14.3. Agilent GC Sampler 80/120 User Manual, Edition 03/2010, Agilent Technologies.

14.4. James C. Garriott (Editor), Medicolegal Aspects of Alcohol, 5th Ed., 2008.

14.5. Moffat, Anthony C. Clarke's Analysis of Drugs and Poisons: In Pharmaceuticals, Body Fluids and Postmortem Material, 4th Ed., London: Pharmaceutical Press, 2004. Print.

14.6. G. Machata, "Determination of Alcohol in Blood by Gas Chromatographic Head Space Analysis," Clinical Chemistry Newsletter, Vol. 4 No. 2 (1972), 29-32.

14.7. Static Headspace Blood Alcohol Analysis with the G1888 Network Headspace Sampler, Agilent Technologies, Inc., 2004, Publication Number 5989-0959EN.

14.8. T. Macchia, et al, "Ethanol in Biological fluids: Headspace GC Measurement," Journal of Analytical Toxicology, Vol. 19 (1975), 241-245.

14.9. G. Machata, "The Advantages of Automated Blood Alcohol Determination by Head Space Analysis," International Journal of Legal Medicine, Vol. 75 (1975), 229-234.

14.10. Agilent OpenLAB CDS ChemStation Edition Concepts and Workflows, Agilent Technologies Inc., 2011, Manual Part Number M8301-90012.

14.11. Caplan, Yale H. and Goldberger, Bruce A. (Editors), Garriott's Medicolegal Aspects of Alcohol, 6th Ed., 2015.

15. Records

15.1. Case file
15.2. Quality control check file
15.3. Calibration file
15.4. GC Instrument log
15.5. GC Maintenance log
15.6. GC Calibration log

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB documents
		Updated section 3. Added wording to line 4.4.4.1. and reference 14.10.
1/16/15	2	Updated for minor additions, deletions, corrections and clarifications: 4.1.5, 4.4.3, 4.4.4, 7.4.1, 8.8.3.2, 9.1.1.2, 9.1.1.3, 9.1.1.4.1, 9.1.2.2, 9.1.2.3, 9.1.2.6.2, 10.1.1.2, 12.1, 14.11

8: Technical Procedure for Enzyme Linked Immunosorbent Assay (ELISA) as a Drug Screen

1. **Purpose / Scope** – This procedure details the use of the Tecan Workstation and Enzyme Linked Immunosorbent Assay to screen blood for controlled substances, controlled substance metabolites and non-controlled substances in the DWI Blood Chemistry Unit of the CCBI Crime Laboratory.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.2. Performance verification – The initial confirmation of the reliability of a previously or externally validated method or instrument.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. ELISA – Enzyme linked immunosorbent assay
   3.3. QCC – Quality control check
   3.4. LOD – Limit of detection

4. **Equipment, Materials and Reagents**

   4.1. Equipment

      4.1.1. Tecan EVO 75/2 liquid handling platform
      4.1.2. Tecan HydroFlex washer
      4.1.3. Tecan Sunrise reader
      4.1.4. Data station and printer

   4.2. Materials

      4.2.1. Vortex mixer
      4.2.2. Test tubes, 12 x 75mm
      4.2.3. Test tube stoppers or caps
      4.2.4. Class A volumetric flask, 10 ml
      4.2.5. Mechanical pipetters: 0.20 ml, 0.250ml, 1mL and 100 µL
      4.2.6. Deionized water

   4.3. Reference Materials

4.3.1. Benzoylecgonine, 1 mg/mL
4.3.2. Carisoprodol, 1 mg/mL
4.3.3. Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (THC-COOH), 1 mg/mL – Diluted 1:1 with methanol for a concentration of 0.5 mg/ml
4.3.4. (±)-Methadone, 1 mg/ml
4.3.5. (±)-Methamphetamine, 1mg/mL
4.3.6. Morphine, 1 mg/mL
4.3.7. Nordiazepam, 1 mg/mL
4.3.8. Phenobarbital, 1 mg/mL
4.3.9. Zolpidem, 1 mg/mL

4.4. Critical Reagents

4.4.1. Negative blood

4.5. Commercial Reagents

4.5.1. Methanol, ACS grade
4.5.2. 1 N HCl, ACS grade
4.5.3. 1 N NaOH, ACS grade
4.5.4. Immunalysis ELISA kits for barbiturates, benzodiazepines, carisoprodol, cocaine metabolite, metabolites of delta-9-THC, methamphetamine, methadone, opiates and zolpidem containing:
4.5.4.1. 96 Well - coated microplates
4.5.4.2. Enzyme conjugate, matched to the microplates
4.5.4.3. TMB substrate reagent
4.5.4.4. Zolpidem TMB substrate reagent
4.5.4.5. Stop reagent

4.6. Prepared Reagents

4.6.1. ELISA Calibration Solution

4.6.1.1. To a 10 mL class A volumetric flask, add the following Reference Materials:

4.6.1.1.1. 0.025 mL Benzoylecgonine, 1 mg/mL
4.6.1.1.2. 0.025 mL Carisoprodol,1 mg/mL
4.6.1.1.3. 0.025 mL Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol, 0.5 mg/mL
4.6.1.1.4. 0.025 mL (±)-Methadone, 1 mg/mL
4.6.1.1.5. 0.050 mL (±)-Methamphetamine, 1 mg/mL
4.6.1.1.6. 0.025 mL Morphine, 1 mg/mL
4.6.1.1.7. 0.025 mL Nordiazepam, 1 mg/mL
4.6.1.1.8. 0.500 mL Phenobarbital, 1 mg/mL

4.6.1.1.9. 0.025 mL Zolpidem, 1 mg/mL

4.6.1.2. Dilute the flask to volume with methanol.

4.6.1.3. Lot number: yyymmdd / ELISACal / Initials

4.6.1.4. Expiration: One year

4.6.1.5. Storage: freezer

4.6.1.6. QCC: performed with each use, refer to 7.23.

4.6.2.ELISA Verification Solution

4.6.2.1. Prepare with Reference Materials from a different supplier or lot than those used in 4.6.1

4.6.2.2. To a 10 mL class A volumetric flask, add the following Reference Materials:

4.6.2.2.1. 0.025 mL Benzoylecgonine, 1 mg/mL
4.6.2.2.2. 0.025 mL Carisoprodol,1 mg/mL
4.6.2.2.3. 0.025 mL Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol, 0.5 mg/mL
4.6.2.2.4. 0.025 mL (±)-Methadone, 1 mg/mL
4.6.2.2.5. 0.050 mL (±)-Methamphetamine, 1 mg/mL
4.6.2.2.6. 0.025 mL Morphine, 1 mg/mL
4.6.2.2.7. 0.025 mL Nordiazepam, 1 mg/mL
4.6.2.2.8. 0.500 mL Phenobarbital, 1 mg/mL
4.6.2.2.9. 0.025 mL Zolpidem, 1 mg/mL

4.6.2.3. Dilute the flask to volume with methanol.

4.6.2.4. Lot number: yyymmdd / ELISAVer / Initials

4.6.2.5. Expiration: One year

4.6.2.6. Storage: freezer

4.6.2.7. QCC: performed with each use, refer to 7.23

5. Performance Verification for New Instruments

5.1. New Tecan workstations shall be installed by a manufacturer representative and shown to meet any manufacturer's requirements.

5.2. Performa a performance verification on new Tecan workstations prior to use for casework according to the CCBI Crime Laboratory Administrative Procedure for Validations and Performance Verifications.

5.3. Maintain the performance verification documentation in the DWI Blood Chemistry Unit.

6. Maintenance

6.1. Record all maintenance in the ELISA maintenance log or ELISA monthly maintenance log at the time it is performed.

6.2. Record any instrument errors or malfunctions in the instrument log at the time they are observed along with any action taken. Notify the DWI Blood Chemistry Unit Technical Leader of any malfunctions that cannot be corrected and mark the instrument log "Out of Service." The Technical Leader shall correct the problem or schedule repair by an approved vendor and mark the instrument log "In Service" when the instrument is returned to service.

6.3. Preventive maintenance shall be performed by an approved vendor annually.

6.4. Monthly maintenance

6.4.1. Empty and fill the two deionized water reservoirs, flush and prime the system.

6.4.2. Perform an~~d~~ acid/base wash of the instrument using 1 N HCl and 1N NaOH.

6.4.2.1. Turn on the computer, workstation, washer, and reader.

6.4.2.2. Open Navitrak 2, Utilities, and select Regular Maintenance. Follow the instrument instruction to place the system intake tube into a beaker containing 250 ml of 1N HCl. The instrument will rinse and soak with the acid solution for 15 minutes.

6.4.2.3. After the 15 minute time period, follow the instrument instruction to place the intake tube into a beaker containing 500 ml of deionized water. The instrument will rinse with deionized water.

6.4.2.4. Follow the instrument instruction to place the system intake tube into a beaker containing 250 ml of 1N NaOH. The instrument will rinse and soak with the base solution for 15 minutes.

6.4.2.5. After the 15 minute time period, follow the instrument instruction to place the intake tube into a beaker containing 500 ml of deionized water. The instrument will rinse with deionized water.

6.4.2.6. Follow the instrument instruction to place the system intake tube into a beaker containing 250 ml of 1N HCl. The instrument will rinse with the acid solution.

6.4.2.7. Follow the instrument instruction to place the system intake tube into the system deionized water container and to conduct a flush / prime of the system.

6.4.2.8. When the flush / prime has completed, exit Navitrak 2 and direct the instrument to return the robotic arms to the home position.

6.4.2.9. Turn off the workstation, reader and computer. Select Night Rinse on the washer.

7. Procedure

7.1. Allow all solutions and samples to equilibrate to room temperature.

7.2. Check system liquid containers and fill with deionized water if necessary. Check the waste containers and empty if necessary.

7.3. Check the printer and add paper if needed.

7.4. Turn on the workstation, washer, reader and computer.

7.5. Open Navitrak 2, Utilities, and select Flush/Prime. The instrument will position the robotic arms and prime / flush the two pipets. When completed, exit Navitrak 2 and direct the instrument to return the robotic arms to the home position. The instrument is now ready to begin analysis of samples.

7.6. Ensure that all blood samples to be analyzed are homogenous by shaking and/or vortexing.

7.6.1. If a homogenous sample cannot be obtained, make a notation in the case file detailing the condition of the sample and its handling.

7.7. Calibration Standard

7.7.1. At the time of analysis, pipet 0.100 mL of the ELISA Calibration Solution into a test tube.

7.7.2. Add 4.9 mL of negative blood to the test tube.

7.7.3. Cap and vortex the test tube.

7.7.4. The concentration of the calibration standard is 1000 ng/mL phenobarbital, 50 ng/mL nordiazepam, 50 ng/mL morphine, 50 ng/mL benzoylecgonine, 50 ng/mL methadone, 25

ng/mL THC-COOH, 100 ng/mL methamphetamine, 50 ng/mL carisoprodol, and 50 ng/mL zolpidem.

7.7.5. Prepare as directed in 7.10. Dispose of any unused portion.

7.8. Verification Standard

7.8.1. At the time of analysis add 0.200 mL of the ELISA Verification Solution to a test tube.

7.8.2. Add 4.8 mL of negative blood to the test tube.

7.8.3. Cap and vortex the test tube.

7.8.4. Prepare as directed in 7.10. Dispose of any unused portion.

7.8.5. The concentration of the verification standard is 2000 ng/mL phenobarbital, 100 ng/mL nordiazepam, 100 ng/mL morphine, 100 ng/mL benzoylecgonine, 100 ng/mL methadone, 50 ng/mL THC-COOH, 200 ng/mL, methamphetamine, 100 ng/mL carisoprodol, and 100 ng/mL zolpidem.

7.9. Negative Standard

7.9.1. Prepare a negative standard as described in 7.10 using negative blood.

7.10. Pipet 0.250 ml of the calibration standard, verification standard, negative standard and each blood sample to be analyzed into each of two labeled disposable glass test tubes using a mechanical pipet labeled "Blood."

7.10.1. Pipette 2.5 ml of deionized water into each of the test tubes.

7.10.2. Cap / stopper the tubes and vortex for approximately 10 seconds.

7.10.3. Examine the tubes and remove any excessive bubbles from the surface of the liquid.

7.11. Arrange the test tubes into the sample racks. The calibration standard, verification standard, negative standard and case samples must be prepared in duplicate and run concurrently. The samples must be arranged in the sample racks in correct order: negative standard duplicates, then calibration standard duplicates, then the verification standard duplicates, followed by the case sample duplicates.

7.12. Arrange the plates with the desired assays wells. If zolpidem is one of the assays, then arrange zolpidem only on plate number one. Plates two through six may contain one or two assays on each plate, depending on the number of samples that are being analyzed.

7.13. When the plates are arranged, place the plates on the workstation deck.

7.14. Place the sample racks containing the negative controls, calibration standards, verification standards and case samples on the workstation deck.

7.15. Fill the appropriate reagent troughs with zolpidem TMB substrate, TMB substrate and stop reagent.

7.16. Fill the screw-top vials with conjugate and place these vials in the conjugate rack in the appropriate order based upon the arrangement of assays on the plates.

7.17. Open Navitrak 2, Test Panel, and configure the plates and assays based upon the arrangement of the plates. Ensure that the placement of the conjugate vials matches the arrangement of the plates.

7.18. Select Sample List and prepare the sample list, listing in order the negatives, calibration standards, verification standards and case samples. Ensure that the placement matches the configuration of the sample list.

7.19. Select Review and ensure that the configuration of the plates, samples and reagents is correct.

7.20. Select Run EVO to begin the analysis.

7.20.1. The workstation automatically pipets the negatives, standards and case samples into the wells on each plate followed by the conjugates.

7.20.2. There is a one hour incubation period.

7.20.3. The plates are then rinsed with deionized water in the washer.

7.20.4. Zolpidem TMB substrate is pipetted into the zolpidem assay wells. TMB substrate is pipetted into the remaining assay wells.

7.20.5. There is a thirty minute incubation period.

7.20.6. The stop acid reagent is pipetted into the wells.

7.20.7. The plates are moved to the reader and the absorbance measured and the data printed.

7.21. After the results of each plate and assay are printed, exit Navitrak 2 and direct the instrument to return the robotic arms to the home position.

7.22. Print the sample list and plate layout. Select Night Rinse on the washer. Turn off the workstation and reader.

7.23. An ~~reciprocal~~ inverse relationship exists between absorbance and concentration.

7.23.1. The adjusted negative standard value, i.e. LOD, negative cut-off, is the average value of the negative standard duplicates minus three standard deviations.

7.23.2. The average of the calibration standard duplicates is the positive cut-off.

7.24. Quality Control

7.24.1. The variation coefficient of the duplicate preparations must be 20% or less. Do not evaluate samples with variation coefficients greater than 20% unless each absorbance value is less than the positive cut-off. Do not evaluate samples for assays with negative, calibration or verification standards with a variation coefficient greater than 20%.

7.24.2. The adjusted negative standard value must be greater than the average of the calibration standard duplicates plus three standard deviations. Do not evaluate samples for assays that do not meet this requirement.

7.24.3. The average of the verification standard duplicates must be less than the average of the calibration standard duplicates, i.e. must be evaluated as positive. Do not evaluate samples for assays that do not meet this requirement.

7.24.4. If the requirements listed in 7.24.1 through 7.24.3 are not met, appropriate action, such as preparation of new solutions, may be taken and the analysis repeated. ~~Refer to the CCBI Crime Laboratory Administrative Procedure for Corrective and Preventive Action~~.

7.25. Record all analyses in the ELISA instrument log with initials, date, ELISAQC file name, refer to 7.28. Record any assays that do not meet the requirements in 7.24 along with the requirement and the observed value.

7.26. The data is exported into an Excel file.

7.26.1. Save the ELISA template file with the file name from 7.28.

7.26.2. Copy the data for each assay from the generated Excel file and paste it into the corresponding assay of the ELISAQC Excel file.

7.26.3. Save the file.

7.26.4. Print the summary and all sample worksheets.

7.27. Evaluate case samples that have an average absorbance value less than or equal to the positive cut-off as positive. Evaluate case samples that have an average absorbance value greater than the positive cut-off, but less than the negative cut-off as elevated. Evaluate case samples that have an average absorbance value equal to or greater than the negative cut-off as negative.

Sample comparison	*Evaluated Result*
Sample average ≥ Negative cut-off	Negative
Negative cut-off > Sample average > Positive cut-off	Elevated
Sample average ≤ Positive cut-off	Positive

7.28. Prepare a file labeled "ELISAQC" followed by the eight digit format date of the analysis "yyymmdd." Multiple analyses on a single date may be indicated be adding unique letters to the end of the file name in alphabetical order.

7.28.1. Maintain this file in the DWI Blood Chemistry Unit.

7.28.2. Include in the file the printed summary worksheet from 7.26.4 and:

7.28.2.1. Lot numbers of the microplates
7.28.2.2. Lot numbers of the enzyme conjugates
7.28.2.3. Lot number of the TMB substrate
7.28.2.4. Lot number of the Zolpidem TMB substrate,
7.28.2.5. Lot number of the stop reagent
7.28.2.6. Lot number of the ELISA calibration solution
7.28.2.7. Lot number of the ELISA verification solution
7.28.2.8. Lot number of the negative blood
7.28.2.9. All sample and standard individual absorbances
7.28.2.10. All sample and standard average absorbances
7.28.2.11. All sample and standard duplicate absorbance standard deviations
7.28.2.12. All sample and standard duplicate absorbance variation coefficients
7.28.2.13. Adjusted negative standard value
7.28.2.14. Calibration standard average absorbance value plus three standard deviations

7.28.3. Reference the file in each sample case file.

7.29. Record in each sample case file the printed sample worksheet from 7.26.4 and:

7.29.1. Reference to the ELISAQC file
7.29.2. Lot numbers of the microplates
7.29.3. Lot numbers of the enzyme conjugates
7.29.4. Lot number of the TMB substrate
7.29.5. Lot number of the Zolpidem TMB substrate,
7.29.6. Lot number of the stop reagent

7.29.7. Lot number of the ELISA calibration solution
7.29.8. Lot number of the ELISA verification solution
7.29.9. Lot number of the negative blood
7.29.10. Case sample and standard individual absorbances
7.29.11. Case sample average absorbance
7.29.12. Case sample evaluation, if evaluated
7.29.13. Standard average absorbances
7.29.14. Case sample and standard duplicate absorbance standard deviations
7.29.15. Case sample and standard duplicate absorbance variation coefficients
7.29.16. Adjusted negative standard value
7.29.17. Calibration standard average absorbance value plus three standard deviations

8. Reporting

8.1. Refer to the DWI Blood Chemistry Unit Technical Procedure for DWI Analysis.

9. Calculations

9.1. Mean, $\bar{x} = \frac{\sum x}{n}$ where n = number of measured values

9.2. Standard deviation, $s = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$

9.3. Variation coefficient, $CV\ (\%) = 100\frac{s}{\bar{x}}$

10. Limitations

10.1. This procedure is a screening test. Additional analysis is required to report the identification of a controlled substance or controlled substance metabolite. Refer to the DWI Blood Chemistry Unit Technical Procedure for DWI Blood Chemistry Analysis.

10.2. Refer to the individual Immunalysis ELISA kit information for cross-reactivities.

10.3. The addition of sodium azide to blood affects the analysis and is not suitable for analysis with this procedure. Interference with the enzyme process may cause false positive and false negative results. Refer to 12.5.

10.4. The data obtained from the ELISA analyzer is displayed with 5 significant figures. Any trailing digits that are zero are not printed by the analyzer software.

11. Safety

11.1. Refer to the Crime Laboratory Safety Manual.

12. References

12.1. Immunalysis Tecan Freedom EVO 75 Workstation Operating Manual V2.2, Tecan Schweiz AG, Switzerland, 2007.

12.2. Instructions for Use for Magellan, V 1.2, Tecan.

12.3. Instructions for Use for Sunrise Microplate Absorbance Reader, V2.0, Tecan.

12.4. Instructions for Use for Hydroflex, V2.2, Tecan.

12.5. Levine, Barry ed., *Principles of Forensic Toxicology*. 3rd edition. AACC Press, 2009, 119-139.

12.6. Immunalysis ELISA kit inserts available from *info@immunalysis.com*. ~~http://www.immunalysis.com/images/stories/labs/cross%20reactivities2.pdf~~

12.7. Moffat, Anthony C. ed. *Clarke's Analysis of Drugs and Poisions,* Volume 1, 4th edition, Pharmaceutical Press, 2011.

13. Records

13.1. ELISA Instrument Log
13.2. ELISA Maintenance Log
13.3. ELISA Monthly Maintenance Log

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3.
1/16/15	2	Updated for minor corrections and clarifications: 4.6.1.1.4, 4.6.1.1.5, 7.23, 7.24.4, 7.27 and 12.6

9: Technical Procedure for Solid Phase Extraction of Basic Drugs for GC-MS Analysis

1. **Purpose / Scope -** The procedure may be used to isolate basic drugs from blood using solid phase extraction for analysis by gas chromatography - mass spectrometry in the DWI Blood Chemistry Unit of the CCBI Crime Laboratory.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. MeCl – Methylene chloride
   3.3. IPA - Isopropanol
   3.4. QCC – Quality control check

4. **Equipment, Materials and Reagents**

   4.1. Equipment

   4.1.1. pH meter
   4.1.2. TurboVap Evaporator
   4.1.3. Mechanical pipettors and corresponding tips
   4.1.4. Volumetric flasks, class A
   4.1.5. Volumetric pipets, class A

   4.2. Materials

   4.2.1. Test tubes, 16 x 125mm and 13 x 100mm
   4.2.2. Test tube caps or stoppers
   4.2.3. Vortex Mixer
   4.2.4. Centrifuge
   4.2.5. UCT Clean Screen DAU Solid Phase Extraction Columns
   4.2.6. Deionized water
   4.2.7. Positive pressure manifold
   4.2.8. Nitrogen, UHP

   4.3. Reagents

4.3.1. Critical Reagents

4.3.1.1. Negative blood
4.3.1.2. BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)

4.3.2. Reference materials

4.3.2.1. Prazepam

4.3.3. Commercial reagents

4.3.3.1. Methylene Chloride, ~~ACS~~ HPLC grade, unstabilized
4.3.3.2. Ammonium Hydroxide, ACS grade
4.3.3.3. Ethyl Acetate, ACS grade
4.3.3.4. Glacial Acetic Acid, ACS grade
4.3.3.5. Methanol, ACS grade
4.3.3.6. Hexane, ACS grade
4.3.3.7. Isopropanol, ACS grade
4.3.3.8. Disodium Hydrogen Phosphate, Anhydrous, ACS grade
4.3.3.9. Sodium Dihydrogen Phosphate, Monohydrate, ACS grade

4.3.4. Prepared Reagents

4.3.4.1. Acetic Acid, 1.0 M

4.3.4.1.1. Add 28.6 mL glacial acetic acid to 400 mL deionized water in a 500 mL volumetric flask.

4.3.4.1.2. Mix and dilute to 500 mL with deionized water.

4.3.4.1.3. Lot Number: Eight digit format year/month/day/AceticAcid1.0M/initials of preparer.

4.3.4.1.4. Expiration: six months

4.3.4.1.5. Storage: closed container

4.3.4.1.6. QCC: Tests acidic with pH or litmus paper

4.3.4.2. Methylene chloride (MeCl) : Isopropanol (IPA) 4:1 with 2 % Ammonium Hydroxide

4.3.4.2.1. Thoroughly mix isopropanol with ammonium hydroxide, add methylene

chloride and mix well.

4.3.4.2.2. Lot number: Eight digit format year/month/day/Amm4:1MeCl:IPA/initials of preparer.

4.3.4.2.3. Expiration: end of day

4.3.4.2.4. Storage: closed container

4.3.4.2.5. QCC: basic to litmus or pH paper

4.3.4.3. 0.1 M Monobasic sodium phosphate, NaH2PO4

4.3.4.3.1. Dissolve 1.38 grams monobasic sodium phosphate monohydrate, in deionized water in a 100 mL volumetric flask.

4.3.4.3.2. Mix and dilute to 100 mL with deionized water.

4.3.4.3.3. Lot number: Eight digit format year/month/day/0.1M_NaH2PO4/initials of preparer

4.3.4.3.4. Expiration: one year

4.3.4.3.5. Storage: closed container

4.3.4.3.6. QCC: Tests acidic with pH or litmus paper

4.3.4.4. 0.1 M Dibasic sodium phosphate, Na2HPO4

4.3.4.4.1. Dissolve 1.42 grams dibasic sodium phosphate, anhydrous, in 80 mL deionized water in a 100 mL volumetric flask.

4.3.4.4.2. Mix and dilute to 100 mL with deionized water.

4.3.4.4.3. Lot number: Eight digit format year/month/day/0.1M_Na2HPO4/initials of preparer.

4.3.4.4.4. Expiration: one year

4.3.4.4.5. Storage: closed container

4.3.4.4.6. QCC: Tests basic with pH paper or litmus paper.

4.3.4.5. 100mM Phosphate Buffer, pH 6.0

4.3.4.5.1. Dissolve 0.85 g Na_2HPO_4 and 6.07 g NaH_2PO_4-H_2O in 400 mL DI water and dilute to 500mL.

4.3.4.5.2. Adjust pH to 6.0 +/- 0.1 with 0.1M monobasic sodium phosphate (lowers pH) or 0.1M dibasic sodium phosphate (raises pH).

4.3.4.5.3. Lot number: Eight digit format year/month/day/0.1MPhosBuffer/initials of preparer

4.3.4.5.4. Storage: refrigerate in closed container

4.3.4.5.5. Expiration: one month

4.3.4.5.6. QCC: Record final pH

4.3.4.6. Prazepam Internal Standard Stock Solution, 100 µg/ml

4.3.4.6.1. Dilute 1.0 ml of a 1.0 mg/ml prazepam reference standard solution to 10 ml in a class A volumetric flask. Mix.

4.3.4.6.2. Lot number: Eight digit format year/month/day/100µg/mlPrazepamISStock/initials of preparer.

4.3.4.6.3. Storage: freezer in closed container

4.3.4.6.4. Expiration: three years

4.3.4.6.5. QCC: N/A, refer to 4.3.4.8.

4.3.4.7. Prazepam Internal Standard Solution, 2µg/ml

4.3.4.7.1. Pipet 1.0 ml Prazepam Internal Standard Stock solution, 100 ng/ml, into a 50 ml class A volumetric flask. Dilute to volume with methanol.

4.3.4.7.2. Lot number: Eight digit format year/month/day/2µg/mlPrazepamIS /initials of preparer

4.3.4.7.3. Storage: refrigerate in closed container

4.3.4.7.4. Expiration: one year

4.3.4.7.5. QCC: successful negative control extraction

5. Quality Control

5.1. Positive control

5.1.1. Prazepam is added to each basic extraction sample as an internal standard. For each basic extraction sample, the mass spectrum of the prazepam internal standard must have a positive comparison to prazepam reference material and the signal-to-noise ratio for the prazepam internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.2. Negative Control

5.2.1. For each extraction batch of blood samples prepare a negative control as directed in Section 6 with 2.0 mL of negative blood.

5.2.2. The mass spectrum of the prazepam internal standard of the negative control must have a positive comparison to prazepam reference material and the signal-to-noise ratio for the prazepam internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.2.3. The negative control must not contain any controlled substances, controlled substance metabolites or any substance being identified in the sample. The negative control shall be subjected to the same post extraction techniques as any case samples in the batch.

6. Procedure

6.1. Allow all solutions and samples to equilibrate to room temperature.

6.2. Ensure that all blood samples are homogenous by shaking and/or vortexing.

6.2.1. If a homogenous sample cannot be obtained, make a notation in the worksheet detailing the condition of the sample and its handling.

6.3. To 1 mL of 100 mM phosphate buffer add 100 µl of the prazepam internal standard solution, mix/vortex.

6.4. Add 2.0 mL of blood sample to be analyzed (case sample or negative blood for the negative control) using a mechanical pipettor labeled “blood” and add 3 mL of deionized water.

6.5. Mix/vortex and let stand for 5 minutes to lyse red blood cells.

6.6. Mix/vortex sample and centrifuge for 10 minutes at >2000 RPM

6.7. Decant liquid portion of the sample into 2 mL of 100 mM phosphate buffer solution.

6.8. Place the Clean Screen DAU Extraction columns into the positive pressure manifold column rack and place the column rack onto the support arms of the manifold.

6.9. Turn on the nitrogen to the manifold and select the corresponding switches on the front and rear of the manifold to allow nitrogen flow to the corresponding columns.

6.10. Adjust the two pressure regulators on the front of the manifold. Use the low pressure regulator to load or aspirate a sample or liquid from the column at a flow rate of 1 to 2 ml per minute, 1 psi or less. Use the high pressure regulator to dry the column with a pressure setting of 80 psi.

6.11. Condition the column with 3 ml methanol.

6.12. Rinse the column with 3 ml 4:1 MeCl:IPA with 2% NH_4OH

6.13. Condition the column with 3 ml deionized water

6.14. Condition the column with 1 ml 100 mM phosphate buffer

6.15. Load sample onto column

6.16. Rinse column with 3 ml deionized water

6.17. Rinse column with 1 ml acetic acid, 1.0 M

6.18. Rinse column with 3 ml methanol

6.19. Dry column for 5 minutes at 80 psi nitrogen

6.20. Collect basic drugs with 3 ml 4:1 MeCl:IPA with 2% NH_4OH.

6.21. Evaporate the solvent from the collection test tube using the TurboVap LV Evaporator, refer to the DWI Blood Chemistry Unit Technical Procedure for Biotage TurboVap LV Evaporator. Remove the tubes immediately upon reaching dryness.

6.22. Reconstitute with 50 µl ethyl acetate or perform silylation as directed in 6.22.1 through 6.22.4 for improved analysis by GC-MS.

6.22.1. Derivatize the extract in the collection tube or transfer to a vial using ethyl acetate and evaporate the ethyl acetate. Discontinue evaporation immediately upon reaching dryness.

6.22.2. Derivatize by adding 50 µl of BSTFA with 1% TMCS to the vial or test tube and capping.

6.22.3. Mix and heat the vial or test tube for 30 minutes at 80°C.

6.22.4. Remove from the heat source and allow the vial or test tube to cool.

6.23. Analyze by GC-MS, refer to the DWI Blood Chemistry Unit Technical Procedure for Gas Chromatography – Mass Spectrometry.

6.24. Record the following in the case file

6.24.1. Lot number of internal standard
6.24.2. Lot number of negative blood
6.24.3. Lot number of UCT Clean Screen DAU Solid Phase Extraction Columns
6.24.4. If applicable, the lot number of BSTFA with 1 % TMCS

7. Limitations

7.1. Refer to the references and other published chemical information as needed to determine the fraction in which a target analyte is expected to elute. Typically barbiturates, carisoprodol, meprobamate and some benzodiazepines elute in the acidic/neutral fraction. Typically alkaloids, amphetamines, opiates, zolpidem, tramadol and most benzodiazepines elute in the basic fraction.

7.2. Refer to the references and other published chemical information as needed to determine the need for derivatization. Typically morphine and benzoylecgonine need to be derivatized for detection by GC-MS. Some benzodiazepines and other substances may need to be derivatized for detection by GC-MS, e.g., a benzodiazepine or opiate ELISA indication and no corresponding substance detected in a non-derivatized sample.

7.3. Do not allow the solid phase extraction columns to dry during the extraction other than at steps indicated.

7.4. Store solid phase extraction columns in a closed container.

8. Safety

8.1. Refer to the CCBI Crime Laboratory Safety Manual

9. References

9.1. UCT Solid Phase Extraction Manual, United Chemical Technologies Inc. Bristol, PA., (2011) 9 – 12, 56 – 58.

9.2. BSTFA with 1 % TMCS Product Specification, Sigma-Aldrich Co, (1997).

9.3. Moffat, Anthony C. ed. *Clarke's Analysis of Drugs and Poisions,* Volume 1, 4th edition, Pharmaceutical Press, 2011.

9.4. O'Neal, Maryadele J. ed. The Merck Index – An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co Inc., Whitehouse Station, NJ, (2006).

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
11/8/13	2	Update to line 7.1 due to incorporation of DBCTP14
1/16/15	3	Updated for minor corrections and changes: 1, 4.3.4.2, 4.3.4.3.1, 4.3.4.4.1, 5.1, 5.2.2 and 6.22.1.
9/21/15	4	Require use of unstabilized HPLC grade methylene chloride

10: Technical Procedure for Solid Phase Extraction of Drugs for GC-MS analysis

1. **Purpose / Scope –** This procedure is used to isolate acidic / neutral drugs from blood, when an acidic / neutral fraction is collected, using solid phase extraction for analysis by gas chromatography – mass spectrometry in the DWI Blood Chemistry Unit of the CCBI Crime Laboratory. This procedure may also be used to isolate basic drugs from blood when a basic fraction is collected.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. IPA – Isopropanol
   3.3. MeCl – Methylene chloride
   3.4. QCC – Quality control check

4. **Equipment, Materials and Reagents**

   4.1. Equipment

      4.1.1. pH meter
      4.1.2. TurboVap Evaporator
      4.1.3. Mechanical pipettors and corresponding tips
      4.1.4. Volumetric flasks, class A
      4.1.5. Volumetric pipets, class A

   4.2. Materials

      4.2.1. Test tubes, 16 x 125mm and 13 x 100mm
      4.2.2. Test tube caps or stoppers
      4.2.3. Vortex Mixer
      4.2.4. Centrifuge
      4.2.5. UCT Clean Screen DAU Solid Phase Extraction Columns
      4.2.6. Deionized water
      4.2.7. Positive pressure manifold
      4.2.8. Nitrogen, UHP

4.3. Reagents

4.3.1. Critical Reagents

4.3.1.1. Negative blood
4.3.1.2. BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)

4.3.2. Reference materials

4.3.2.1. Hexobarbital
4.3.2.2. Prazepam

4.3.3. Commercial reagents

4.3.3.1. Methylene Chloride, ~~ACS~~ HPLC grade, unstabilized
4.3.3.2. Ammonium Hydroxide, ACS grade**,**
4.3.3.3. Ethyl Acetate, ACS grade
4.3.3.4. Glacial Acetic Acid, ACS grade
4.3.3.5. Methanol, ACS grade
4.3.3.6. Hexane, ACS grade
4.3.3.7. Isopropanol, ACS grade
4.3.3.8. Disodium Hydrogen Phosphate, Anhydrous, ACS grade
4.3.3.9. Sodium Dihydrogen Phosphate, Monohydrate, ACS grade

4.3.4. Prepared Reagents

4.3.4.1. Acetic Acid, 1.0 M

4.3.4.1.1. Add 28.6 mL glacial acetic acid to 400 mL deionized water in a 500 mL volumetric flask.

4.3.4.1.2. Mix and dilute to 500 mL with deionized water.

4.3.4.1.3. Lot Number: Eight digit format year/month/day/AceticAcid1.0M/initials of preparer.

4.3.4.1.4. Expiration: six months

4.3.4.1.5. Storage: closed container

4.3.4.1.6. QCC: Tests acidic with pH or litmus paper

4.3.4.2. Methylene chloride (MeCl) : Isopropanol (IPA) 4:1 with 2 % Ammonium Hydroxide

4.3.4.2.1. Thoroughly mix isopropanol with ammonium hydroxide, add methylene chloride and mix well.

4.3.4.2.2. Lot number: Eight digit format year/month/day/Amm4:1MeCl:IPA/initials of preparer.

4.3.4.2.3. Expiration: end of day

4.3.4.2.4. Storage: closed container

4.3.4.2.5. QCC: basic to litmus or pH paper

4.3.4.3. 0.1 M Monobasic sodium phosphate, NaH2PO4

4.3.4.3.1. Dissolve 1.38 grams monobasic sodium phosphate, monohydrate, in deionized water in a 100 mL volumetric flask.

4.3.4.3.2. Mix and dilute to 100 mL with deionized water.

4.3.4.3.3. Lot number: Eight digit format year/month/day/0.1M_NaH2PO4/initials of preparer

4.3.4.3.4. Expiration: one year

4.3.4.3.5. Storage: closed container

4.3.4.3.6. QCC: Tests acidic with pH or litmus paper

4.3.4.4. 0.1 M Dibasic sodium phosphate, Na2HPO4

4.3.4.4.1. Dissolve 1.42 grams dibasic sodium phosphate, anhydrous, in 80 mL deionized water in a 100 mL volumetric flask.

4.3.4.4.2. Mix and dilute to 100 mL with deionized water.

4.3.4.4.3. Lot number: Eight digit format year/month/day/0.1M_Na2HPO4/initials of preparer.

4.3.4.4.4. Expiration: one year

4.3.4.4.5. Storage: closed container

4.3.4.4.6. QCC: Tests basic with pH paper or litmus paper.

4.3.4.5. 100mM Phosphate Buffer, pH 6.0

4.3.4.5.1. Dissolve 0.85 g Na_2HPO_4 and 6.07 g NaH_2PO_4-H_2O in 400 mL DI water and dilute to 500mL.

4.3.4.5.2. Adjust pH to 6.0 +/- 0.1 with 0.1M monobasic sodium phosphate (lowers pH) or 0.1M dibasic sodium phosphate (raises pH).

4.3.4.5.3. Lot number: Eight digit format year/month/day/0.1MPhosBuffer/initials of preparer

4.3.4.5.4. Storage: refrigerate in closed container

4.3.4.5.5. Expiration: one month

4.3.4.5.6. QCC: Record final pH

4.3.4.6. Hexobarbital Internal Standard. 40 µg/ml

4.3.4.6.1. Dilute 1 ml of a 1.0 mg/mL hexobarbital reference material solution to volume in a 25 ml class A volumetric flask with methanol. Mix.

4.3.4.6.2. Lot number: Eight digit format year/month/day/40µg/mlHexIS/initials of preparer.

4.3.4.6.3. Storage: refrigerator

4.3.4.6.4. Expiration: one year

4.3.4.6.5. QCC: successful negative control extraction

4.3.4.7. Prazepam Internal Standard Stock Solution, 100 µg/ml

4.3.4.7.1. Dilute 1.0 ml of a 1.0 mg/ml prazepam reference standard solution to 10 ml in a class A volumetric flask. Mix.

4.3.4.7.2. Lot number: Eight digit format year/month/day/100µg/mlPrazepamISStock/initials of preparer.

4.3.4.7.3. Storage: freezer in closed container

4.3.4.7.4. Expiration: three years

4.3.4.7.5. QCC: N/A, refer to 4.3.4.8.

4.3.4.8. Prazepam Internal Standard Solution, 2µg/ml

4.3.4.8.1. Pipet 1.0 ml Prazepam Internal Standard Stock solution, 100 ng/ml, into a 50 ml class ~~calss~~ A volumetric flask. Dilute to volume with methanol.

4.3.4.8.2. Lot number: Eight digit format year/month/day/2µg/mlPrazepamIS /initials of preparer

4.3.4.8.3. Storage: refrigerate in closed container

4.3.4.8.4. Expiration: one year

4.3.4.8.5. QCC: successful negative control extraction

5. Quality Control

5.1. Positive control

5.1.1. Hexobarbital is added to each acidic / neutral extraction sample as an internal standard. For each acidic / neutral extraction sample, the mass spectrum of the hexobarbital internal standard must have a positive comparison to hexobarbital reference material and the signal-to-noise ratio for the hexobarbital internal standard gas chromatographic peak must be 5 ~~2~~:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.1.2. Prazepam is added to each basic extraction sample as an internal standard. For each basic extraction sample, the mass spectrum of the prazepam internal standard must have a positive comparison to prazepam reference material and the signal-to-noise ratio for the prazepam internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.2. Negative Control

5.2.1. For each extraction batch of blood samples prepare a negative control as directed in Section 6 with 2.0 mL of negative blood.

5.2.2. The mass spectrum of the hexobarbital internal standard of the negative control must have a positive comparison to hexobarbital reference material and the signal-to-noise ratio for the hexobarbital internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷

the response at the baseline or valley immediately before the internal standard signal.

5.2.2.1. For each basic fraction the mass spectrum of the prazepam internal standard of the negative control must have a positive comparison to prazepam reference material and the signal-to-noise ratio for the prazepam internal standard gas chromatographic peak must be 5 ~~2~~:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.2.3. The negative control must not contain any controlled substances, controlled substance metabolites or any substance being identified in the sample. The negative control shall be subjected to the same post extraction techniques as any case samples in the batch.

6. Procedure

6.1. Allow all solutions and samples to equilibrate to room temperature.

6.2. Ensure that all blood samples are homogenous by shaking and/or vortexing.

6.2.1. If a homogenous sample cannot be obtained, make a notation in the worksheet detailing the condition of the sample and its handling.

6.3. To 1 mL of 100 mM phosphate buffer add 50 µl of the hexobarbital internal standard solution. If a basic fraction is to be collected, add 100 µl of the prazepam internal standard solution. Mix/vortex.

6.4. Add 2.0 mL of blood sample to be analyzed (case sample or negative blood for the negative control) using a mechanical pipettor labeled "blood" and add 3 mL of deionized water.

6.5. Mix/vortex and let stand for 5 minutes to lyse red blood cells.

6.6. Mix/vortex sample and centrifuge for 10 minutes at >2000 RPM

6.7. Decant liquid portion of the sample into 2 mL of 100 mM phosphate buffer solution.

6.8. Place the Clean Screen DAU Extraction columns into the positive pressure manifold column rack and place the column rack onto the support arms of the manifold.

6.9. Turn on the nitrogen to the manifold and select the corresponding switches on the front and rear of the manifold to allow nitrogen flow to the corresponding columns.

6.10. Adjust the two pressure regulators on the front of the manifold. Use the low pressure regulator to load or aspirate a sample or liquid from the column at a flow rate of 1 to 2 ml per minute, 1 psi or less. Use the high pressure regulator to dry the column with a pressure setting of

80 psi.

6.11. Condition the column with 3 ml methanol.

6.12. Rinse the column with 3 ml 4:1 MeCl:IPA with 2% NH_4OH

6.13. Condition the column with 3 ml deionized water

6.14. Condition the column with 1 ml 100 mM phosphate buffer

6.15. Load sample onto column

6.16. Rinse column with 3 ml deionized water

6.17. Rinse column with 1 ml acetic acid, 1.0 M

6.18. Dry column for 5 minutes with nitrogen

6.19. Wash the column with 2 mL of hexane

6.20. Collect acidic / neutral drugs with 6 ml methylene chloride

6.20.1. To also collect a basic fraction:

6.20.1.1. Rinse column with 3 ml methanol

6.20.1.2. Dry column for 2 minutes at 80 psi nitrogen

6.20.1.3. Collect basic drugs with 3 ml 4:1 MeCl:IPA with 2% NH_4OH in a separate collection tube

6.21. Evaporate the solvent from the collection test tube using the TurboVap LV Evaporator, refer to the DWI Blood Chemistry Unit Technical Procedure for Biotage TurboVap LV Evaporator. Remove the tubes immediately upon reaching dryness.

6.22. Reconstitute acidic / neutral fractions with 100 µl ethyl acetate or perform silylation as directed in 6.22.1. through 6.22.4. for improved analysis by GC-MS. Acidic / neutral fractions may be diluted with additional ethyl acetate to achieve improved chromatography. Do not dilute the negative control fraction with more ethyl acetate than used for the sample fraction. Record the amount of ethyl acetate used. Reconstitute basic fractions with 50 µl ethyl acetate or perform silylation as directed in 6.22.1 through 6.22.4 for improved analysis by GC-MS.

6.22.1. Derivatize the extract in the collection tube or transfer to a vial using ethyl acetate and evaporate the ethyl acetate. Discontinue evaporation immediately upon reaching dryness.

6.22.2. Derivatize by adding 50 µl of BSTFA with 1% TMCS to the vial or test tube and capping.

6.22.3. Mix and heat the vial or test tube for 30 minutes at 80°C.

6.22.4. Remove from the heat source and allow the vial or test tube to cool.

6.23. Analyze by GC-MS, refer to the DWI Blood Chemistry Unit Technical Procedure for Gas Chromatography – Mass Spectrometry.

6.24. Record the following in the case file

6.24.1. Lot number of internal standard(s)
6.24.2. Lot number of negative blood
6.24.3. Lot number of UCT Clean Screen DAU Solid Phase Extraction Columns
6.24.4. If applicable, the lot number of BSTFA with 1 % TMCS

7. Limitations

7.1. Refer to the references and other published chemical information as needed to determine the fraction in which a target analyte is expected to elute. Typically barbiturates, carisoprodol, meprobamate and some benzodiazepines elute in the acidic/neutral fraction. Typically alkaloids, amphetamines, opiates, zolpidem, tramadol and most benzodiazepines elute in the basic fraction.

7.2. Refer to the references and other published chemical information as needed to determine the need for derivatization. Typically morphine and benzoylecgonine need to be derivatized for detection by GC-MS. Some benzodiazepines and other substances may need to be derivatized for detection by GC-MS, e.g., an opiate or benzodiazepine ELISA indication and no corresponding substance detected in a non-derivatized sample.

7.3. Do not allow the solid phase extraction columns to dry during the extraction other than at steps indicated.

7.4. Store solid phase extraction columns in a closed container.

8. Safety

8.1. Refer to the CCBI Crime Laboratory Safety Manual

9. References

9.1. UCT Solid Phase Extraction Manual, United Chemical Technologies Inc. Bristol, PA., (2011) 9 – 12, 56 – 58.

9.2. BSTFA with 1 % TMCS Product Specification, Sigma-Aldrich Co, (1997).

9.3. Moffat, Anthony C. ed. *Clarke's Analysis of Drugs and Poisions,* Volume 1, 4th edition, Pharmaceutical Press, 2011.

9.4. O'Neal, Maryadele J. ed. The Merck Index – An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co Inc., Whitehouse Station, NJ, (2006).

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
11/8/13	2	Incorporation of DBCTP14
1/16/15	3	Updated for minor corrections and additions: 1, 4.3.4.2, 4.3.4.3.1, 4.3.4.4.1, 4.3.4.7.1, 4.3.4.9.1, 5.1.1, 5.1.2, 5.2.2, 6.3 and 6.22.1.
9/21/15	4	Require use of unstabilized HPLC grade methylene chloride

11: Technical Procedure for Solid Phase Extraction of THC and THC-COOH for GC-MS Analysis

1. **Purpose / Scope –** This procedure is used to isolate delta-9-tetrahydrocannabinol and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid from blood using United Chemical Technologies styre screen solid phase extraction columns for analysis by gas chromatography - mass spectrometry in the DWI Blood Chemistry Unit of the CCBI Crime Laboratory.

2. Definitions

2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

3. Abbreviations

3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
3.2. THC - *delta*-9-tetrahydrocannabinol
3.3. THC-D_3 - *delta*-9-tetrahydrocannabinol-D_3
3.4. THC_COOH - 11-nor-*delta*-9-tetrahydrocannabinol-9-carboxylic acid
3.5. THC-COOH-D_3 - 11-nor-*delta*-9-tetrahydrocannabinol-9-carboxylic acid-D_3
3.6. QCC – Quality control check

4. Equipment, Materials and Reagents

4.1. Equipment

4.1.1. TurboVap Evaporator
4.1.2. Mechanical pipettors and corresponding tips
4.1.3. Volumetric flasks, class A
4.1.4. Volumetric pipets, class A

4.2. Materials

4.2.1. Test tubes, 16 x 125mm and 13 x 100mm
4.2.2. Test tube caps or stoppers
4.2.3. Vortex mixer
4.2.4. Centrifuge
4.2.5. UCT Styre Screen solid phase extraction columns
4.2.6. Deionized water
4.2.7. Positive pressure manifold
4.2.8. Nitrogen, UHP

4.3. Reagents

4.3.1. Critical Reagents

4.3.1.1. Negative blood
4.3.1.2. BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)

4.3.2. Reference materials

4.3.2.1. *delta*-9-tetrahydrocannabinol
4.3.2.2. 11-nor-*delta*-9-tetrahydrocannabinol-9-carboxylic acid
4.3.2.3. *delta*-9-tetrahydrocannabinol-D_3 (THC-D_3)
4.3.2.4. 11-nor-*delta*-9-tetrahydrocannabinol-9-carboxylic acid-D_3 (THC-COOH-D_3)

4.3.3. Commercial reagents

4.3.3.1. Ammonium Hydroxide, ACS grade**,**
4.3.3.2. Ethyl Acetate, ACS grade
4.3.3.3. Glacial Acetic Acid, ACS grade
4.3.3.4. Acetonitrile, ACS grade
4.3.3.5. Hexane, ACS grade

4.3.4. Prepared Reagents

4.3.4.1. Cannabinoid Column Rinse Reagent

4.3.4.1.1. Mix deionized water, acetonitrile and ammonium hydroxide in a ratio of 84:15:1.

4.3.4.1.2. Lot Number: Eight digit format year/month/day/CanRinse/initials of preparer.

4.3.4.1.3. Expiration: end of day

4.3.4.1.4. Storage: closed container

4.3.4.1.5. QCC: Successful positive control

4.3.4.2. Cannabinoid Elution Reagent

4.3.4.2.1. Mix hexane, ethyl acetate and glacial acetic acid in a ratio of 49:49:2.

4.3.4.2.2. Lot Number: Eight digit format year/month/day/CanElute/initials of preparer.

4.3.4.2.3. Expiration: end of day

4.3.4.2.4. Storage: closed container

4.3.4.2.5. QCC: Successful positive control

4.3.4.3. Cannabinoid Internal Standard

4.3.4.3.1. Prepare a solution that contains 1.0 µg/ml of THC-D_3 reference material and THC-COOH-D_3 reference material.
Example: Pipet 1.0 ml of a 100 µg/ml THC-D_3 reference material solution and 1.0 ml of a 100 µg/ml THC-COOH-D_3 reference material solution to a 100 ml volumetric flask and dilute to volume with methanol.

4.3.4.3.2. Lot Number: Eight digit format year/month/day/CanIS/initials of preparer.

4.3.4.3.3. Expiration: one year

4.3.4.3.4. Storage: refrigerator

4.3.4.3.5. QCC: Successful calibration

4.3.4.4. Cannabinoid Calibration Solution

4.3.4.4.1. Prepare a solution that contains 1.0 µg/ml of THC and THC-COOH.
Example: Pipet 1.0 ml of a 100 µg/ml THC reference material solution and 1.0 ml of a 100 µg/ml THC-COOH reference material solution to a 100 ml volumetric flask and dilute to volume with methanol.

4.3.4.4.2. Lot Number: Eight digit format year/month/day/CanCal/initials of preparer.

4.3.4.4.3. Expiration: one year

4.3.4.4.4. Storage: refrigerator

4.3.4.4.5. QCC: Successful calibration

4.3.4.5. Cannabinoid Verification Solution

4.3.4.5.1. Prepare this solution with reference materials from a different supplier or different supplier lot than those used in 4.3.4.4.

4.3.4.5.2. Prepare a solution that contains 1.0 µg/ml of THC and THC-COOH. Example: Pipet 1.0 ml of a 100 µg/ml THC reference material solution and 1.0 ml of a 100 µg/ml THC-COOH reference material solution to a 100 ml volumetric flask and dilute to volume with methanol.

4.3.4.5.3. Lot Number: Eight digit format year/month/day/CanVer/initials of preparer.

4.3.4.5.4. Expiration: one year

4.3.4.5.5. Storage: refrigerator

4.3.4.5.6. QCC: Successful calibration

5. Calibration

5.1. Calibrate the GC-MS CANSIM data analysis method upon preparation of a new lot of Cannabinoid Internal Standard Solution and after instrument maintenance that may affect the calibration as determined by the DWI Blood ~~Drug~~ Chemistry Technical Leader.

5.2. Calibration Standards

5.2.1. Negative Cannabinoid Calibration Standard

5.2.1.1. Use 1.0 mL of negative blood.

5.2.1.2. Prepare as directed in Section 7.

5.2.2. 10 ng/mL Cannabinoid Calibration Standard

5.2.2.1. In duplicate, add 10 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.2.2.2. Prepare each duplicate as directed in Section 7.

5.2.3. 20 ng/mL Cannabinoid Calibration Standard

5.2.3.1. In duplicate, add 20 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.2.3.2. Prepare each duplicate as directed in Section 7.

5.2.4.50 ng/mL Cannabinoid Calibration Standard

5.2.4.1. In duplicate, add 50 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.2.4.2. Prepare each duplicate as directed in Section 7.

5.2.5.100 ng/mL Cannabinoid Calibration Standard

5.2.5.1. In duplicate, add 100 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.2.5.2. Prepare each duplicate as directed in Section 7.

5.2.6.150 ng/mL Cannabinoid Calibration Standard

5.2.6.1. In duplicate, add 150 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.2.6.2. Prepare each duplicate as directed in Section 7.

5.2.7.200 ng/mL Cannabinoid Calibration Standard

5.2.7.1. In duplicate, add 200 µL of the Cannabinoid Calibration Solution to 1.0 mL of negative blood.

5.2.7.2. Prepare each duplicate as directed in Section 7.

5.3. Verification Standards

5.3.1. Negative Cannabinoid Verifier

5.3.1.1. Use 1.0 mL of negative blood.

5.3.1.2. Prepare as directed in Section 7.

5.3.2.20 ng/mL Cannabinoid Verifier

5.3.2.1. Add 20 µL of the Cannabinoid Verification Solution to 1.0 mL of negative blood.

5.3.2.2. Prepare as directed in Section 7.

5.3.3.50 ng/mL Cannabinoid Verifier

5.3.3.1. Add 50 µL of the Cannabinoid Verification Solution to 1.0 mL of negative blood.

5.3.3.2. Prepare as directed in Section 7.

5.3.4. 150 ng/mL Cannabinoid Verifier

5.3.4.1. Add 150 µL of the Cannabinoid Verification Solution to 1.0 mL of negative blood.

5.3.4.2. Prepare as directed in Section 7.

5.4. Chromatograph and quantitate the calibration standards and verification standards on the GC-MS using the CANSIM GC-MS method with the negative cannabinoid calibration standard first and the negative cannabinoid verification standard following the 200 ng/ml cannabinoid calibration standard.

5.5. Save the data analysis method according to the format "CANSIM" and eight digit format year/month/day.

Example: CANSIM20130101

5.6. Update the calibration with the calibration samples.

5.6.1. The response at each level shall be the response of the calibration standard at that level.

5.6.2. The retention time for each component shall be the average retention time of the 50 ng/mL calibration standard duplicates.

5.6.3. The ion ratios for each component shall be the average ion ratios of the 50 ng/mL calibration standard duplicates.

5.6.4. The calibration curve will be fitted to a linear model.

5.6.5. Save the method.

5.7. Evaluate according to the acceptance criteria:

5.7.1. The coefficient of determination, r^2, must be 0.995 or greater for each component.

5.8. Reprocess the calibration samples against the curve. Evaluate according to the acceptance criteria:

5.8.1. The quantitation results must be within +/- 20% of the target concentration.

5.8.2. The qualifier ion ratio must be within +/- 20% of the 50 ng/ml ion ratio.
5.8.3. The retention time of each component must be within +/- 0.5% of the 50 ng/ml retention time.
5.8.4. The negative calibration standard must not contain THC or THC-COOH.

5.9. If the calibration does not meet the acceptance criteria, appropriate action, e.g., maintenance or new solution preparation, shall be taken and the calibration repeated.

5.10. Save the data analysis method.

5.11. Calibration Verification

5.11.1. Quantitate the cannabinoid verification standards with the data analysis method from 5.5. Evaluate according to the acceptance criteria:

5.11.1.1. THC and THC-COOH must not be identified in the negative verification standard.

5.11.1.2. The retention times of the THC, THC-COOH and the internal standards must not differ by more than 2.0 % from the target value.

5.11.1.3. The qualifier ion ratios of THC, THC-COOH and the internal standards must be within +/- 20 % of the target value.

5.11.1.4. The quantitation results of each component must be within +/- 20 % of the target value.

5.11.2. If the calibration does not meet the acceptance criteria, appropriate action, e.g., maintenance or new solution preparation, shall be taken and the calibration repeated.

5.12. Prepare a file folder labeled with the name of the calibrated method, refer to 5.5. Maintain this file in the DWI Blood Chemistry Unit. This Calibration file shall include:

5.12.1. Lot number of the cannabinoid calibration solution.
5.12.2. Lot number of the cannabinoid verification solution.
5.12.3. Lot number and expiration date of the cannabinoid internal standard.
5.12.4. Lot number of negative blood.
5.12.5. Quantitation reports for each calibration and verification standard.
5.12.6. Printed calibration table.
5.12.7. Each component r^2 value and the equation describing each calibration curve.

5.13. Record each calibration in the instrument log with the date, lot number of internal standard used, internal standard expiration date and operator initials.

6. Quality Control

6.1. Internal standards are added to each cannabinoid extraction sample. The internal standards must be identified in each extraction sample.

6.2. Positive Control

6.2.1. For each extraction batch at least one verification standard must be extracted as a positive control.

6.2.1.1. The quantitation results must be within +/- 20% of the target concentration.
6.2.1.2. The qualifier ion ratio must be within +/- 20% of the target ratio.
6.2.1.3. The retention time of each component must be within +/- 0.5% of the target retention time.

6.3. Negative Control

6.3.1. For each extraction batch a negative verification standard must be extracted as a negative control.

6.3.1.1. The negative blood must not contain THC or THC-COOH.

7. Procedure

7.1. For the case samples, positive control(s), negative control(s), and an additional 50 ng/ml verification standard - allow all solutions and samples to equilibrate to room temperature.

7.2. Ensure that all blood samples are homogenous by shaking and/or vortexing.

7.2.1. If a homogenous sample cannot be obtained, make a notation in the worksheet detailing the condition of the sample and its handling.

7.3. Add 1.0 mL of blood sample to be analyzed using a mechanical pipettor labeled "blood" to a test tube.

7.4. Slowly add 100 µL of the cannabinoid internal standard solution.

7.5. Slowly add 2 mL of cold acetonitrile with vortexing.

7.6. Mix/Vortex samples and let stand for 5 minutes.

7.7. Mix/Vortex samples.

7.8. Centrifuge for 10 minutes at >2000 RPM**.**

7.9. Decant liquid portion of the sample into a clean test tube and evaporate to approximately 200 µl.

7.10. Add 2.0 mL of deionized water.

7.11. Place the Styre Screen Extraction columns into the column rack and place the column rack onto the support arms of the manifold.

7.12. Turn on the nitrogen to the manifold and select the corresponding switches on the front and rear of the manifold to allow nitrogen flow to the corresponding columns - one switch per row of columns.

7.13. Adjust the two pressure regulators on the front of the manifold. The low pressure regulator is used to load or aspirate a sample or liquid from the column at a flow rate of 1-2 ml per minute (1 psi or less). The high pressure regulator is used to dry the column with a pressure setting of 80 psi.

7.14. Load sample onto column.

7.15. Rinse column with 1.0 mL of the cannabinoid column rinse reagent.

7.16. Dry column for 15 minutes.

7.17. Collect cannabinoids with 3 mL of the cannabinoid elution reagent.

7.18. Evaporate the solvent from the collection test tube using the TurboVap LV Evaporator at 40°C, refer to the DWI Blood Chemistry Unit Technical Procedure for Biotage TurboVap LV Evaporator. Remove the tubes immediately upon reaching dryness.

7.19. Add 50 µl ethyl acetate and derivatize by adding 50 µl BSTFA with 1% TMCS to the vial or collection test tube and capping. Mix and heat the vial or collection test tube at 70° C for 20 minutes. Remove from heat source and allow the vial or collection test tube to cool before analysis.

7.20. Analyze by GC-MS using the CANSIM method, refer to the DWI Blood Chemistry Unit Technical Procedure for Gas Chromatography – Mass Spectrometry.

7.21. Quantitate and print the 50 ng/ml verification standard using the current CANSIM calibrated method.

7.22. Save the most current calibrated CANSIM method with the Drug Chemist initials and the date added to the end of the method name. The calibrated method must correspond to the lot number of Cannabinoid Internal Standard Solution used to prepare the samples.

7.23. Use the 50 ng/ml verification standard to replace the qualifier ion ratios and retention times of

each component. The updated values are the target values.

7.24. Save the updated method as directed in 7.22.

7.25. Quantitate the remaining extracts and injection blanks using the updated method.

7.26. Prepare a file folder labeled "CanQCC" followed by the date in yyyymmdd format. Maintain this file in the DWI Blood Chemistry Unit. Include in the QCC file:

7.26.1. Lot number of the cannabinoid verification solution.
7.26.2. Lot number and expiration date of the cannabinoid internal standard.
7.26.3. Lot number of negative blood.
7.26.4. Quantitation reports for the 50 ng/ml verification standard, positive control(s), negative control(s) and corresponding blank injections.
7.26.5. Printed calibration table of the updated method.
7.26.6. Each component r^2 value and the equation describing each calibration curve of the updated method

7.27. Record in the case file:

7.27.1. Reference to the QCC file, refer to 7.26
7.27.2. Sample quantitation report and corresponding blank injection
7.27.3. Lot number and expiration of the cannabinoid internal standard solution
7.27.4. Lot numbers of the cannabinoid column rinse reagent and elution reagent

8. Limitations

8.1. While the CANSIM method provides quantitative results, the method is for SIM mass spectral comparison and GC retention time comparison only. The quantitative results shall not be reported. Refer to the Solid Phase Extraction of THC and THC-COOH for GC-MS Analysis validation data in the DWI Blood Chemistry Unit or the GC-MS Relative Retention Time reference collection for limit of detection and limit of quantitation.

9. Safety

9.1. Refer to the CCBI Crime Laboratory Safety Manual

10. References

10.1. Solid Phase Extraction Applications Manual, 2011, United Chemical Technologies Inc., Bristol, PA. pp. 40-41.

10.2. BSTFA with 1 % TMCS Product Specification, Sigma-Aldrich Co, (1997).

11. Records

11.1. CanQCC file
11.2. CANSIM calibration file
11.3. Case file

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3.
1/16/15	2	Corrected reference to technical leader

12: Technical Procedure for Gas Chromatography/Mass Spectrometry (GC-MS)

1. **Purpose / Scope** - This procedure provides direction for the initial setup, performance checks and usage of gas chromatograph – mass spectrometer instruments in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification Crime Laboratory.

2. **Definitions**

   2.1. Performance verification - The initial confirmation of the reliability of a previously or externally validated method or instrument.
   2.2. Quality control check - Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
   2.3. Reference Material – Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. QCC – Quality Control Check
   3.3. GC – Gas chromatograph
   3.4. MS – Mass spectrometer
   3.5. MSD - Mass Selective Detector
   3.6. TIC – Total ion chromatogram
   3.7. RT – Retention time
   3.8. RRT – Relative retention time

4. **Equipment, Materials and Reagents**

   4.1. Equipment

      4.1.1. Agilent 7890 gas chromatograph with Agilent 5975 series mass selective detector with Agilent automatic liquid sampler and tray
      4.1.2. Computer with Agilent Analytical MSD Productivity ChemStation software and printer

   4.2. Reference Materials

      4.2.1. Multi-component drug solution containing alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam and temazepam
      4.2.2. Prazepam

4.2.3. Hexobarbital

4.3. Materials

4.3.1. Sample vials, caps and inserts
4.3.2. ALS Syringe, 10µl straight, fixed needle, 23/42/HP
4.3.3. DB5-MS column, 30 m X 0.250 mm X 0.25 µm for AUTO and CANSIM methods
4.3.4. DB5-MS column, 12 m X 0.200 mm X 0.33 µm for 70TOX1 method
4.3.5. Agilent inlet liner, splitless, single taper with glass wool, deactivated, 900 µl, 4mm ID
4.3.6. Agilent liner O-ring
4.3.7. Merlin microseal
4.3.8. Septum wrench
4.3.9. Tweezers
4.3.10. Clean, lint free, non-nylon gloves
4.3.11. Wrenches, ¼ inch and ½ inch
4.3.12. Gold plated inlet seal with cross and 0.375 outer diameter washer
4.3.13. Star or Torx screwdriver
4.3.14. Flat head screwdriver, large
4.3.15. Hex key, 5 mm
4.3.16. Inland 45 pump oil
4.3.17. Funnel
4.3.18. Hex ball driver, 1.5 mm
4.3.19. Hex ball driver, 2.0 mm
4.3.20. Wrench, open-end, 10 mm
4.3.21. Alumina abrasive powder
4.3.22. Cotton swabs
4.3.23. Ultrasonic bath

4.4. Commercial Reagents

4.4.1. Methanol, Optima grade
4.4.2. Ethyl acetate, Optima grade
4.4.3. Helium gas, Grade 5.0
4.4.4. Perfluorotributylamine [PFTBA], neat

4.5. Prepared Reagents

4.5.1. Monthly QCC solution

4.5.1.1. Mix hexobarbital reference material, prazepam reference material and benzodiazpine reference material solution from 4.2.1 with ethyl acetate to yield a solution containing 10 ng/µl of each component.

4.5.1.2. Lot Number: Eight digit format year/month/day/BenzoMix/initials of preparer.

4.5.1.3. Expiration: one year

4.5.1.4. Storage: closed container in freezer

4.5.1.5. QCC: Successful monthly QCC

5. Standards and Controls

5.1. A GC-MS logbook shall be maintained near the instrument. The logbook shall contain the GC-MS Activity Log, GC-MS Daily QCC Log, the GC-MS Monthly QCC Log, the GC-MS Maintenance Logs and any manufacturer's certificates, performance verification documentation, QCC printouts and maintenance documentation.

5.1.1. GC-MS Activity Log

5.1.1.1. Record the date, sequence identification, initials of operator, and any comments for each sequence analyzed on the DWI Blood Chemistry GC-MS Activity Log.

5.1.1.2. Upon completion of each sequence, print the sequence log and maintain in the logbook.

5.1.1.3. Record any error messages on the DWI Blood Chemistry GC-MS Activity Log.

5.1.2. GC-MS Daily QCC log

5.1.2.1. Record all Daily QCC's on the Daily QCC log with the date, initials, and results and any comments.

5.1.2.2. Place Daily QCC printouts in the instrument logbook.

5.1.3. Monthly QCC log

5.1.3.1. Record all Monthly QCC's on the Monthly QCC log with the date, initials, results and any comments, refer to 5.7.

5.1.3.2. Place Monthly QCC printouts in the instrument logbook.

5.1.4. Archive the instrument logbook yearly.

5.1.4.1. Label the instrument logbook with the instrument serial number and year and store near the instrument.

5.2. When the GC-MS has been placed out of service for non-routine maintenance, malfunction or leaving direct control of the Laboratory the DWI Blood Chemistry Technical Leader shall evaluate the instrument and determine if any additional quality control checks are needed to ensure instrument performance. At a minimum, a Daily QCC must be successfully performed prior to placing the instrument back in service, refer to 5.6.

5.2.1. If maintenance is performed that may affect retention times, a monthly QCC, refer to 5.7., shall be performed before the instrument is placed back in service.

5.3. The Blood Drug Chemist shall record any malfunctions or error messages in the GC-MS Activity Log, notify the DWI Blood Chemistry Technical Leader of any malfunctions or error messages and place the instrument out of service by marking the GC-MS Activity Log "Out of Service."

5.4. The DWI Blood Chemistry Technical Leader shall correct any problems with the instrument or request service. The DWI Blood Chemistry Technical Leader shall examine the effect(s), if any, of a malfunction or error message on analysis results and implement the CCBI Laboratory Procedure for Corrective and Preventive Action as required.

5.5. Negative Quality Control Check

5.5.1. Negative QCC's are performed prior to each sample injection, refer to 7.3.

5.6. Daily Quality Control Check – AutoTune

5.6.1. Perform an Autotune (atune) with Perfluorotributylamine (PFTBA) as the tuning standard prior to beginning the first sample sequence each day the instrument is in use.

5.6.1.1. Sample sequences that continue overnight may be allowed to complete without performing a new tune provided that they do not extend more than twenty-four hours beyond the time of the tune or noon, whichever is later.

5.6.2. Compare the atune report to previous ones. Record any major variations on the atune report and on the Daily QCC log and notify the DWI Blood Chemistry Technical Leader.

5.6.3. The mass assignments of the three tuning masses in the upper part of the report must be within ±- 0.2 amu of 69.00, 219.00, and 502.00.

5.6.4. The peak widths of the three tuning masses must be within ± 0.10 amu of 0.60 and the peaks must generally be smooth and symmetrical.

5.6.5. There are no target abundances, the system optimizes sensitivity across the entire mass range. It is normal at times to have a base peak of 219 instead of 69. The relative abundance ratio of mass 502 to mass 69 must be within must be greater than 3 %.

5.6.6. The 70/69 isotopic ratio must be from 0.5 – 1.6, the 220/219 ratio must be from 3.2 – 5.4, and the 503/502 the ratio must be from 7.9 – 12.3.

5.6.7. If any parameter listed in 5.5.2. - 5.5.6. does not meet the requirements listed, document the deviation on the atune ~~stune~~ report and on the GC-MS daily QCC log.

5.6.7.1. Perform another atune.

5.6.7.2. If the problem persists, place the instrument out of service by marking the activity log "out of service" and notify the DWI Blood Chemistry Technical Leader.

5.6.7.3. The DWI Blood Chemistry Technical Leader shall correct any problems with the instrument or request service. The DWI Blood Chemistry Technical Leader shall examine the effect(s), if any, on analysis results and implement the CCBI Laboratory Procedure for Corrective and Preventive Action as required.

5.6.7.4. The daily QCC must be successfully completed prior to placing the instrument back in service.

5.6.8. The abundance of any peaks less than 69 amu must not be greater than 10 % of the abundance of the base peak.

5.6.8.1. Peaks at 18, 28 or 32 amu are indicative of water, nitrogen and oxygen, respectively, and may indicate an air leak. Other peaks may indicate gas impurities.

5.6.8.2. If an air leak is detected, isolate the leak and tighten fittings to correct the leak and perform another atune.

5.6.8.3. If the problem persists, place the instrument out of service by marking the activity log "out of service" and notify the DWI Blood Chemistry Technical Leader.

5.6.8.4. The DWI Blood Chemistry Technical Leader shall correct any problems with the instrument or request service. The DWI Blood Chemistry Technical Leader shall examine the effect(s), if any, on analysis results and implement the CCBI Laboratory Procedure for Corrective and Preventive Action as required.

5.6.8.5. The daily QCC must be successfully completed prior to placing the instrument back in service.

5.6.9. Initial the atune report and mark any parameter that does not meet the requirement specified. Place the atune report in the instrument logbook and record the results in the GC-MS daily QCC log.

5.7. Monthly Quality Control Check

5.7.1. The multi-component drug solution from 4.5.1 ~~4.2.1.~~ shall be injected on the method corresponding to each instrument, either "AUTO" or "70TOX1," refer to 7.1., each month the instrument is in use to verify instrument performance.

5.7.2. The solution shall, when feasible, be run during the first seven calendar days of each month.

5.7.2.1. If the standard solution is not run during the first seven calendar days of the month, the instrument shall be out of service until the standard solution is successfully run.

5.7.3. Name the multi-component reference material solution data files with "MC" followed by the numerical year and month designation. Name the corresponding solvent blank with the same designation followed by "-b", indicating that it is a blank.

5.7.4. Visually examine the TIC of the monthly QCC solution for chromatographic quality and resolution. All components must exhibit visually symmetrical peaks that are visually baseline resolved or for peaks separated by 0.2 minutes or less, visually resolved at half height. Abundances should be comparable to those of the previous monthly QCC.

5.7.5. Perform a GC relative retention time comparison, refer to 7.7.2., for temazepam and alprazolam.

5.7.6. Perform a mass spectral comparison, refer to 7.7.1., for temazepam and alprazolam.

5.7.7. Each component specified must have a positive comparison for 5.7.5. and 5.7.6.

5.7.7.1. Record any component that does have a positive comparison on the instrument monthly QCC log, place the instrument out of service by marking the GC-MS activity log "out of service" and notify the DWI Blood Chemistry Technical Leader.

5.7.7.2. The DWI Blood Chemistry Technical Leader shall correct any problems with the instrument or request service. The DWI Blood Chemistry Technical Leader shall examine the effect(s), if any, on analysis results and implement the CCBI Laboratory Procedure for Corrective and Preventive Action as required.

5.7.7.3. The monthly QCC must be successfully completed prior to placing the instrument back in service.

5.7.8. Print the monthly QCC TIC with the retention times displayed, the mass spectrum of each component and the corresponding blank TIC.

5.7.8.1. Mark each page with initials and date and note any problems.

5.7.8.2. Record the lot number ~~and supplier~~ of the ~~standard~~ solution on the TIC.

5.7.8.3. File the generated data in the instrument logbook.

5.7.8.4. Record the monthly check in the monthly QCC log.

5.8. Performance Verification for New Instrumentation

5.8.1. New GC-MS instruments shall be installed by a manufacturer representative or approved vendor according to the manufacturer's guidelines.

5.8.2. Prior to use, perform daily QCC's, refer to 5.6., on three separate days. The daily QCC's must meet all specified requirements.

5.8.3. Prior to use, analyze the multi-component drug solution from 4.2.1 on each method (refer to 7.1.) on three separate days.

5.8.3.1. The mass spectra of each component must have a positive mass spectral comparison to reference material, refer to 7.7.1.

5.8.3.2. The relative retention times of each component must have a positive GC retention time comparison to reference material, refer to 7.7.2.

5.8.4. Label the instrument printouts with the lot number of the reference material, initials and date. Record the performance verification in the GC-MS logbook and place the printouts in the GC-MS logbook.

5.8.5. The performance verification must be reviewed and approved by the DWI Blood Chemistry Technical Leader prior to the instrument being used for casework. The DWI Blood Chemistry Technical Leader shall record the review and approval in the GC-MS logbook.

6. Maintenance

6.1. Place the instrument out of service prior to performing any maintenance, other than wash vial and syringe maintenance, by marking the GC-MS activity log "Out of Service." When the instrument is ready to be returned to service, mark the GC-MS activity log "Back in Service."

6.2. Record all maintenance one of the GC-MS maintenance logs at the time it is performed with the name of the person performing the maintenance or repairs, the initials of the Blood Drug Chemist recording the maintenance or repairs, the date, a description of the maintenance or repairs and a list of any parts replaced.

6.3. Record lengths of column trimmed in the activity log and a maintenance log. If the column is trimmed, the instrument shall be out of service until a monthly QCC is successfully completed, refer to 5.7.

6.3.1. The DWI Blood Chemistry Technical Leader shall update the instrument log when the instrument is ready to be used for casework and file any generated data in the instrument logbook located near the instrument.

6.4. Suggested Routine maintenance – The routine maintenance schedule is a suggested guideline. The maintenance schedule will be determined by the DWI Blood Chemistry Technical Leader based upon instrument usage and performance.

6.4.1. Wash Vials

6.4.1.1. Rinse and fill daily when in use. Use methanol in the first wash vial and ethyl acetate in the second wash vial.
6.4.1.2. Record on the GCMS Daily Maintenance Log.
6.4.1.3. Required post-maintenance check: None.

6.4.2. Syringe

6.4.2.1. Inspect monthly for cleanliness and ease of movement. Replace as needed.

6.4.2.1.1. Mount the injector on a parking post.
6.4.2.1.2. Open the injector door.
6.4.2.1.3. Slide the syringe carriage to the top position.
6.4.2.1.4. Completely loosen the plunger thumb screw until it reaches the stop, and lift the plunger carrier off of the syringe plunger.
6.4.2.1.5. Open the syringe latch by swinging it in a counterclockwise direction.
6.4.2.1.6. Carefully pull the top of the syringe out of the flange guide, then lift the needle out of the needle support foot.
6.4.2.1.7. Carefully pass the new syringe needle through the guide hole in the needle support foot.
6.4.2.1.8. Align the syringe flange with the flange guide and press the syringe into place, keeping the needle end in the guide hole of the needle support foot. Make sure that the flat edge of the syringe flange faces out.
6.4.2.1.9. Close the syringe latch by swinging it clockwise until it snaps in place.
6.4.2.1.10. Slide the plunger carrier down until it is completely over the syringe plunger, and tighten the plunger thumb screw until finger- tight.
6.4.2.1.11. Manually move the plunger carrier up and down. If the syringe plunger does not move along with the carrier, repeat the previous steps until installed correctly. Be sure the plunger thumb screw is secure and tight. Verify that the needle is inside the guide hole of the needle support foot. The needle should be

straight and pass freely through the needle guide hole. If the needle is bent or is outside the guide hole, remove the syringe and reinstall.

6.4.2.1.12. Close the injector door.

6.4.2.1.13. Mount the injector on the inlet.

6.4.2.2. Record on the GCMS Monthly Maintenance Log.

6.4.2.3. Required post-maintenance check: None.

6.4.3. Liner

6.4.3.1. Replace monthly, at a minimum, when in use.

6.4.3.1.1. Press [Oven] and set the oven to 35°C. When the temperature reaches setpoint, turn the oven off. Press [Front Inlet] and turn off the inlet temperature and pressure.

6.4.3.1.2. Be careful - The inlet fittings may be hot enough to cause burns. Flip the inlet open.

6.4.3.1.3. Remove liner with tweezers, being careful not to break the liner.

6.4.3.1.4. Hold the new liner with tweezers or lint free tissue and place the o-ring on the liner about 2 to 3 mm from its top end.

6.4.3.1.5. Insert the liner straight down into the inlet and press gently to ensure it is seated.

6.4.3.1.6. Replace the inlet cover and flip the top into place.

6.4.3.1.7. Using ChemStation load a method to return the GC to appropriate settings. If prompted, do not save any method changes.

6.4.3.1.8. Allow the GC to return to the setpoints.

6.4.3.2. Record on the GCMS Monthly Maintenance Log.

6.4.3.3. Required post-maintenance check: Successful daily QCC, refer to 5.6.

6.4.4. Pump Oil

6.4.4.1. Change every six months.

6.4.4.1.1. Vent the MSD by selecting the vent option in Instrument Control of Chemstation. Allow the vent cycle to run, when the cycle is complete and the temperatures are below 100 degrees Celsius turn off the MSD.

6.4.4.1.2. Press [Oven] and set the oven to 35°C. Press [Front Inlet] and turn off the inlet temperature and pressure. When the temperature reaches the setpoint turn the GC off.

6.4.4.1.3. Place a book or other object approximately two inches thick under the pump motor to tilt it up slightly.

6.4.4.1.4. Remove the fill cap.

6.4.4.1.5. Place a container under the drain plug.

6.4.4.1.6. Remove the drain plug. Allow the pump oil to drain out. The foreline pump and oil may be hot.
6.4.4.1.7. Replace the drain plug.
6.4.4.1.8. Remove the book or object used to prop up the pump.
6.4.4.1.9. Refill the foreline pump with Inland 45 pump oil, using a funnel, until the oil level is between the two fill marks in the site window, approximately 0.28 L of oil.
6.4.4.1.10. Wait a few minutes for the oil to settle. If the oil level drops, add oil to bring the oil level near the upper line.
6.4.4.1.11. Reinstall the fill cap.
6.4.4.1.12. Power on the GC.
6.4.4.1.13. Ensure that the vent valve is closed. Holding the MSD chamber door closed, power on the MSD and ensure that the turbo pump speed climbs to 100%.
6.4.4.1.14. Start the Chemstation software and apply setpoints.
6.4.4.1.15. Allow the instrument to equilibrate for two hours prior to tuning.

6.4.4.2. Record on the GCMS Biannual Maintenance Log.
6.4.4.3. Required post-maintenance check: Successful daily QCC, refer to 5.6.

6.4.5. Clean Source

6.4.5.1. Clean annually, at a minimum.

6.4.5.1.1. Vent the MSD by selecting the vent option in Instrument Control of Chemstation. Allow the vent cycle to run, when the cycle is complete and the temperatures are below 100 degrees Celsius turn off the MSD.
6.4.5.1.2. Press [Oven] and set the oven to 35°C. Press [Front Inlet] and turn off the inlet temperature and pressure. When the temperature reaches the setpoint turn the GC off.
6.4.5.1.3. Open the vent valve
6.4.5.1.4. Detach the ribbon cables from the circuit board on the MSD chamber door.
6.4.5.1.5. Pull open the MSD chamber door by hand.
6.4.5.1.6. Detach the leads from the ion source, loosen the screws and remove the ion source.
6.4.5.1.7. Remove the filaments using a hex ball driver.
6.4.5.1.8. Separate the repeller assembly from the source body. The repeller assembly includes the source heater assembly, repeller, and related parts.
6.4.5.1.9. Remove the repeller.
6.4.5.1.10. Unscrew the interface socket. A 10-mm open-end wrench fits the flats on the interface socket.
6.4.5.1.11. Remove the setscrew for the lenses.
6.4.5.1.12. Push the lenses out of the source body.

6.4.5.1.13. If insulators are dirty, clean them with a cotton swab dampened with reagent-grade methanol. If that does not clean the insulators, replace them. Do not abrasively or ultrasonically clean the insulators.

6.4.5.1.14. The filaments and source heater assembly cannot be cleaned ultrasonically. Replace these components if major contamination occurs.

6.4.5.1.15. Collect the following parts that contact the sample or ion beam to be cleaned.

6.4.5.1.15.1. Repeller
6.4.5.1.15.2. Interface socket
6.4.5.1.15.3. Source body
6.4.5.1.15.4. Drawout plate
6.4.5.1.15.5. Drawout cylinder
6.4.5.1.15.6. Ion focus lens
6.4.5.1.15.7. Entrance lens

6.4.5.1.16. Abrasively clean the surfaces that contact the sample or ion beam.

6.4.5.1.17. Use an abrasive slurry of alumina powder and methanol on a cotton swab. Use enough force to remove all discolorations. Polishing the parts is not necessary; small scratches will not harm performance. Also abrasively clean the discolorations where electrons from the filaments enter the source body.

6.4.5.1.18. Rinse away all abrasive residue with reagent-grade methanol.

6.4.5.1.19. Make sure all abrasive residue is rinsed way before ultrasonic cleaning. If the methanol becomes cloudy or contains visible particles, rinse again.

6.4.5.1.20. Separate the parts that were abrasively cleaned from the parts that were not abrasively cleaned.

6.4.5.1.21. Ultrasonically clean the parts (each group separately) for 15 minutes in each of the following solvents: methylene chloride followed by acetone followed by methanol.

6.4.5.1.22. Place the parts in a clean beaker. Loosely cover the beaker with clean aluminum foil (dull side down).

6.4.5.1.23. Dry the cleaned parts in an oven at 100 °C for 5–6 minutes.

6.4.5.1.24. Let the parts cool before you handle them.

6.4.5.1.25. Take care to avoid recontaminating cleaned and dried parts. Put on new, clean gloves before handling the parts. Do not set the cleaned parts on a dirty surface. Set them only on clean, lint-free cloths.

6.4.5.1.26. Slide the drawout plate and the drawout cylinder into the source body.

6.4.5.1.27. Assemble the ion focus lens, entrance lens, and lens insulators.

6.4.5.1.28. Slide the assembled parts into the source body.

6.4.5.1.29. Install the setscrew that holds the lenses in place.

6.4.5.1.30. Reinstall the repeller, repeller insulators, washer, and repeller nut into the source heater assembly. The resulting assembly is called the repeller assembly.

6.4.5.1.31. Reconnect the repeller assembly to the source body. The repeller assembly includes the source heater assembly, repeller, and related parts.

6.4.5.1.32. Reinstall the filaments, replace if excessively worn.

6.4.5.1.33. Reinstall the interface socket.

6.4.5.1.34. Do not overtighten the repeller nut or the ceramic repeller insulators will break when the source heats up. The nut should only be finger-tight.
6.4.5.1.35. Do not overtighten the interface socket. Overtightening could strip the threads.
6.4.5.1.36. Reinstall the ion source into the MSD and reattach the leads.
6.4.5.1.37. Close the vent valve.
6.4.5.1.38. Push the MSD chamber door close and reattach the ribbon cables to the circuit board.
6.4.5.1.39. Power on the GC.
6.4.5.1.40. Holding the MSD chamber door closed, power on the MSD and ensure that the turbo pump speed climbs to 100%.
6.4.5.1.41. Start the Chemstation software and apply setpoints.
6.4.5.1.42. Allow the instrument to equilibrate for two hours prior to tuning.

6.4.5.2. Record on the GCMS Annual Maintenance Log.
6.4.5.3. Required post-maintenance check: Successful daily QCC, refer to 5.6., and monthly QCC, refer to 5.7.

6.4.6. Gold Seal

6.4.6.1. Replace annually, at a minimum.

6.4.6.1.1. Vent the MSD by selecting the vent option in Instrument Control of Chemstation. Allow the vent cycle to run, when the cycle is complete and the temperatures are below 100 degrees Celsius turn off the MSD.
6.4.6.1.2. Press [Oven] and set the oven to 35°C. Press [Front Inlet] and turn off the inlet temperature and pressure. When the temperature reaches the setpoint turn the GC off.
6.4.6.1.3. Be careful - The inlet fittings may be hot enough to cause burns. Loosen the inlet column nut with the ¼ inch wrench and remove the column from the inlet. Cap the open end of the column to prevent contamination.
6.4.6.1.4. Remove the insulation cup from around the base of the inlet using the star screwdriver.
6.4.6.1.5. Use the 1/2-inch wrench to loosen the reducing nut, and then remove it.
6.4.6.1.6. The washer and seal are inside the reducing nut. Remove them, noting their orientation.
6.4.6.1.7. Handle the new gold seal and washer with clean, lint-free, non-nylon gloves. Place the washer in the reducing nut. Place the new inlet base seal on top of it with the raised portion facing down.
6.4.6.1.8. Replace the reducing nut. Use the 1/2-inch wrench to tighten the nut.
6.4.6.1.9. Replace the column and the insulation cup.
6.4.6.1.10. Using ChemStation load a method to return the GC to appropriate settings. If prompted, do not save any method changes.
6.4.6.1.11. Allow the GC to return to the setpoints.

6.4.6.1.12. Ensure that the vent valve is closed. Holding the MSD chamber door closed, power on the MSD and ensure that the turbo pump speed climbs to 100%.
6.4.6.1.13. Start the ChemStation software and apply setpoints.
6.4.6.1.14. Allow the instrument to equilibrate for two hours prior to tuning.

6.4.6.2. Record on the GCMS Annual Maintenance Log.
6.4.6.3. Required post-maintenance check: Successful daily QCC, refer to 5.6., and monthly QCC, refer to 5.7.

7. Procedure

7.1. Instrument Settings

7.1.1. Select a GC-MS method based upon the sample to be analyzed.

7.1.1.1. The AUTO method is used for acidic / neutral solid phase extraction fractions.

7.1.1.1.1. DB5-MS column, 30 m X 0.250 mm X 0.25 µm
7.1.1.1.2. Initial Temperature 70 ºC hold for 1.00 minute
7.1.1.1.3. 70 ºC - 125 ºC @ 40 ºC/minute hold for 1.00 minute
7.1.1.1.4. 125 ºC - 285 ºC @ 17 ºC/minute hold for 12.00 minutes
7.1.1.1.5. Total time of run: 24.79 minutes
7.1.1.1.6. Scan Range 40 – 500
7.1.1.1.7. 230 °C source temperature
7.1.1.1.8. 150 °C quadrupole temperature

7.1.1.2. The 70TOX1 method is used for basic solid phase extraction fractions.

7.1.1.2.1. DB5-MS column, 12 m X 0.200 mm X 0.33 µm
7.1.1.2.2. Initial temperature - 70 ºC hold for 1.00 minute
7.1.1.2.3. 70 ºC - 125 ºC @ 40 ºC/minute hold for 1.00 minute
7.1.1.2.4. 125 ºC - 285 ºC @ 17 ºC/minute hold for 12.00 minutes
7.1.1.2.5. Total time of run: 24.79 minutes
7.1.1.2.6. Scan Range 40 – 500
7.1.1.2.7. 230 °C source temperature
7.1.1.2.8. 150 °C quadrupole temperature

7.1.1.3. The CANSIM method is a selected ion monitoring method used for THC and THC-COOH solid phase extraction samples.

7.1.1.3.1. DB5-MS column, 30 m X 0.250 mm X 0.25 µm
7.1.1.3.2. Initial Temperature 150 ºC hold for 1 minute
7.1.1.3.3. 150 ºC - 235 ºC @ 50 ºC/minute hold for 2.00 minutes

7.1.1.3.4. 235 ºC - 300 ºC @ 15 ºC/minute hold for 5.97 minutes
7.1.1.3.5. Total time of run: 15.00 minutes
7.1.1.3.6. 230 °C source temperature
7.1.1.3.7. 150 °C quadrupole temperature
7.1.1.3.8. SIM ions:
7.1.1.3.8.1. THC quantitative: 386, qualitative 303
7.1.1.3.8.2. THC-d3 quantitative: 389, qualitative 306
7.1.1.3.8.3. THC-COOH quantitative 371, qualitative 473
7.1.1.3.8.4. THC-COOH-d3 quantitative 374, qualitative 476

7.1.1.4. The 70TOX1Deriv method is used for derivatized basic solid phase extraction fractions. It has the same parameters as the 70TOX1 method except for an extended scan range to accommodate increased masses.

7.1.1.4.1. DB5-MS column, 12 m X 0.200 mm X 0.33 µm
7.1.1.4.2. Initial temperature - 70 ºC hold for 1.00 minute
7.1.1.4.3. 70 ºC - 125 ºC @ 40 ºC/minute hold for 1.00 minute
7.1.1.4.4. 125 ºC - 285 ºC @ 17 ºC/minute hold for 12.00 minutes
7.1.1.4.5. Total time of run: 24.79 minutes
7.1.1.4.6. Scan Range 40 – 550
7.1.1.4.7. 230 °C source temperature
7.1.1.4.8. 150 °C quadrupole temperature

7.1.1.5. Each method washes the syringe 10 times between injections to ensure sample integrity

7.1.1.6. When the standard methods are not appropriate to analyze a compound, a modified method may be used in accordance with the CCBI Administrative Procedure for Exceptions.

7.2. Shutdown / Startup

7.2.1. The GC-MS shall be left on at all times.

7.2.2. The computer may be shut down or restarted if needed.

7.2.3. A successful daily QCC check, refer to 5.6., must be performed following any GC or MS shutdown.

7.2.4. Record any shutdown on the GC-MS activity.

7.3. For AUTO and 70TOX1 samples prepared in ethyl acetate, Place a blank ethyl acetate injection in the sequence immediately before each sample injection as a negative QCC using the same method as the sample. Additional ethyl acetate blank injections may be placed in the sequence.

For AUTO, ~~and~~ 70TOX1, 70TOX1Deriv samples derivatized with BSTFA 1% TMCS place a blank BSTFA 1% TMCS injection in the sequence immediately before each sample injection as the negative QCC using the same method as the sample.

7.3.1. Evaluate the negative QCC to ensure that the instrument is free of any controlled substance, any substance being identified in the sample and any substance that may interfere with the identification of sample component(s).

7.3.1.1. Record any negative QCC's that do not meet the requirement in the GC-MS activity log, in the case file and follow the CCBI Laboratory Procedure for Corrective and Preventive Action as required.

7.3.1.2. Note the presence of large amounts of common gas chromatography peaks (e.g., siloxanes) in the GC-MS activity log and notify the DWI Blood Chemistry Technical Leader.

7.4. Use the current date in the names of sequences.

7.5. Include the entire CCBI assigned case file number in the data file name and any additional information needed to uniquely identify the sample.

7.5.1. Data files associated with casework and performance checks shall not be deleted or overwritten.

7.5.2. The DWI Blood Chemistry Technical Leader shall archive data files annually and label with the instrument serial number and dates. Store archived files near the instrument.

7.5.3. Notify the DWI Blood Chemistry Technical Leader if the data storage location becomes full.

7.6. The GC-MS provides retention time data and mass spectral data.

7.7. For full scan methods evaluate the chromatogram and spectra for each peak of interest.

7.7.1. Mass Spectral Comparison

7.7.1.1. For a positive mass spectral comparison, the sample mass spectrum must be substantially comparable, i.e., equivalent, to that of primary or secondary reference material.

7.7.1.2. Record in the case file the mass spectrum of the reference material with the supplier and lot number or other DWI Blood Chemistry Unit designation. Library search results may be included.

7.7.2. GC Relative Retention Time (RRT) Comparison

7.7.2.1. For a positive RRT comparison, the percent difference between the sample retention time relative to the internal standard must be within ± 5 % of a primary or secondary reference material relative to the internal standard. Round relative retention times to the hundredths, refer to 11.7. for rounding instructions. The retention time of the collected mass spectrum may be used to determine the relative retention time.

7.7.2.2. Record in the case file

7.7.2.2.1. A reference to the current RRT chart used, refer to the DWI Blood Chemistry Unit Technical Procedure for Quality Assurance.
7.7.2.2.2. Sample TIC with the retention time(s) displayed or mass spectrum with retention time displayed.
7.7.2.2.3. The percent difference of the reference material and sample relative retention times.

7.7.3. Record in the case record the:

7.7.3.1. Total Ion Chromatogram (TIC) for the sample and corresponding blank.
7.7.3.1.1. Mass spectra of significant peaks of interest, including internal standard
7.7.3.1.2. Expanded mass spectra of any phenethylamines.
7.7.3.2. Batch Negative Control TIC and corresponding blank TIC
7.7.3.2.1. Batch Negative Control internal standard mass spectrum
7.7.3.2.2. Extraction ion chromatograms of batch negative control showing absence of any substance identified in sample.

7.8. For the CANSIM method evaluate the quantitation report

7.8.1. For a positive SIM mass spectral comparison of THC and THC-COOH, the qualifier ion ratio must be within ± 20 % of the target ratio.
7.8.2. For a positive GC retention time comparison, the retention time of the quantifier ion must be within ± 0.5 % of the target retention time.

8. Calculations

8.1. RRT Percent Difference Calculation, round to one decimal place, refer to 11.7.:

|[(reference material RT ÷ reference material internal standard RT) – (sample RT ÷ sample internal standard RT)]| ÷ (reference material RT ÷ reference material internal standard RT) * 100

9. Limitations

9.1. The GC-MS methods described in this procedure shall not be used to distinguish between optical isomers.

9.2. While the CANSIM method provides quantitative results, the method is for SIM mass spectral comparison and GC retention time comparison only. The quantitative results shall not be reported.

9.3. Due to the possibility of conversion of methadone to EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, an inactive metabolite of methadone) during GC/MS analysis do not report EDDP in the presence of methadone.

10. Safety

10.1. Refer to the CCBI Crime Laboratory Safety Manual.

10.2. Handle syringes with care to avoid punctures.

10.3. Use extreme caution handling/installing/removing/transporting compressed gas cylinders. Cylinders shall not be moved without the cylinder cap securely in place.

10.4. Gas Chromatograph and Mass Spectrometer may be extremely hot. Avoid touching hot areas and wear protective gloves while performing maintenance.

11. References

11.1. Agilent 7890A GC User Information, Agilent Instrument Utilities V B.1.06.11343.1852.

11.2. Agilent 5975 MSD User Information, Agilent Instrument Utilities V B.1.06.11343.1852.

11.3. Agilent 7693A ALS User Information, Agilent Instrument Utilities V B.1.06.11343.1852.

11.4. Moffat, A. C., et al., eds. *Clarke's Isolation and Identification of Drugs.* 2nd Edition. London: Pharmaceutical Press, 1986.

11.5. Moffat, A.C., et al., eds. *Clarke's Analysis of Drugs and Poisons.* 4rd Edition. London: Pharmaceutical Press, 2011.

11.6. Pfleger, Maurer, and Weber, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*; 3rd Ed., Vols. 1-2, 2007.

11.7. *Guide for the Use of the International System of Units (SI).* NIST Special Publication 811, 2008 Ed., (March 2008; 2nd printing November 2008). p.43

11.8. Skoog, Douglas A., F. James Holler and Timothy A. Nieman. *Principles of Instrumental Analysis*. 5th Edition. Garcourt Brace & Company, 1998.

11.9. *Agilent GC-MSD ChemStation and Instrument Operation Student Manual Course Number H4043A Volume 1,* Revision E.02.xx. Agilent Technologies: printed February 2008.

11.10. Agilent 5975 Series MSD Operation Manual. 3rd Edition. Agilent Technologies Inc., 2010, Manual Part Number G3170-90036.

11.11. Agilent G1701EA GC/MSD Chemstation Getting Started. 1st Edition. Agilent Technologies Inc., 2011, Manual Part Number G1701-90069.

12. Records

12.1. GC-MS Daily Maintenance Log
12.2. GC-MS Monthly Maintenance Log
12.3. GC-MS Biannual Maintenance Log
12.4. GC-MS Annual Maintenance Log
12.5. GC-MS Non-routine Maintenance Log
12.6. GC-MS Daily QCC Log
12.7. GC-MS Monthly QCC Log
12.8. GC-MS Activity Log
12.9. Case file

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
		Updated section 3 and line 5.6.5. Added lines 11.10 and 11.11.
1/16/15	2	Additions, minor corrections and clarifications in 4.2.2, 4.2.3, 4.5, 5.3, 5.6.7, 5.7.1, 5.7.4, 5.7.8.2, 6.2, 6.4.3.1, 6.4.5.1, 6.4.6.1, 7.1.1.4, 7.3 and 9.3

13: Technical Procedure for DWI Blood Chemistry Analysis

1. Purpose/Scope - This procedure provides direction for analysis of blood in DWI submissions in the DWI Blood Chemistry Unit of the Raleigh/Wake City-County Bureau of Identification.

2. Definitions

2.1. Alcohol – Any substance containing any form of alcohol, including ethanol, methanol, propanol, and isopropanol. (NCGS 20-4.01.(1a))

2.2. Alcohol Concentration – The concentration of alcohol in a person, expressed either as:

a. Grams of alcohol per 100 milliliters of blood; or

b. Grams of alcohol per 210 liters of breath.

The results of a defendant's alcohol concentration determined by a chemical analysis of the defendant's breath or blood shall be reported to the hundredths. Any result between hundredths shall be reported to the next lower hundredth. (NCGS 20-4.01.(1b))

2.3. Impairing Substance - Alcohol, controlled substance under Chapter 90 of the General Statutes, any other drug or psychoactive substance capable of impairing a person's physical or mental faculties, or any combination of these substances. (NCGS §20-4.01.(14a))

3. Abbreviations

3.1. Common chemical terminology and unit abbreviations may be used
3.2. approx. – approximately
3.3. BAC – Blood alcohol concentration
3.4. conc - concentration
3.5. c̄ – containing
3.6. d – dated
3.7. diff – difference
3.8. DWI – Driving while impaired
3.9. ELISA – Enzyme linked immunosorbent assay
3.10. ext – extract / extraction
3.11. GCMS, GC-MS, or GC/MS – gas chromatography–mass spectrometry
3.12. hs – heat sealed
3.13. i – initialed
3.14. init – initial
3.15. LTC – labeled to contain
3.16. me – manila envelope
3.17. MS – mass spectrum
3.18. NIST – National Institute of Standards and Technology
3.19. NQCC – negative quality control check
3.20. NCS – no controlled substances
3.21. p – page

3.22. pg – page
3.23. pb – plastic bag
3.24. pos - positive
3.25. PQCC – positive quality control check
3.26. prelim(s) – preliminary testing(s)
3.27. QCC – quality control check
3.28. rgt – reagent
3.29. rec'd – received
3.30. ref – reference
3.31. RRT – relative retention time
3.32. RT – retention time
3.33. ret'd - returned
3.34. sch – schedule
3.35. std(s) – standard(s)
3.36. s - sealed
3.37. temp – temperature
3.38. tran(s) – transfer(s)
3.39. u or us - unsealed
3.40. w/ or $\bar{w}$ – with
3.41. wt – weight
3.42. zpb – Ziploc plastic bag

4. Equipment, Materials and Reagents

4.1. Refer to the DWI Blood Chemistry Unit Technical Procedures

5. Procedure

5.1. Laboratory facilities are subjected to environmental monitoring, refer to the DWI Blood Chemistry Unit Technical Procedure for General Laboratory Equipment.

5.2. Store DWI submissions of blood evidence in a refrigerator at 4^0C ($\pm$ 3^0C).

5.2.1. Retrieve blood evidence from the Evidence Receiving Unit.

5.2.1.1. Once received by a Blood Drug Chemist, blood evidence shall be maintained in the sole care and custody of the receiving Blood Drug Chemist and stored in their assigned refrigerator.

5.2.1.2. Blood Drug Chemist's shall maintain their assigned refrigerators so that evidence in the process of analysis is stored in a manner that is visually separate from evidence upon which analysis has been completed. Physical barriers need not be implemented to separate in-process evidence from completed evidence.

5.2.2. When blood evidence is removed from the evidence refrigerator for analysis, it shall be maintained in the sole care and custody of the Blood Drug Chemist and be returned to the evidence refrigerator as soon as practicable.

5.2.2.1. When an individual with evidence in their immediate custody leaves the work area during the workday for a short time (e.g., restroom break, meal break) the evidence in the work area must be secured by locking the door to the work area.

5.2.2.2. When evidence is to be left unattended for an extended period (i.e., longer than a meal break) the evidence must be secured by returning it to Blood Drug Chemist's assigned Evidence Refrigerator.

5.2.3. Once analysis of submitted evidence has commenced, it should not be maintained in the in-process portion of the Blood Drug Chemist's assigned Evidence Refrigerator for longer than is reasonably necessary to complete the analysis.

5.2.4. Disposition

5.2.4.1. Blood evidence will be retained for a minimum of twelve months after the report is published.

5.2.4.2. The blood tubes may be destroyed by disposal as biohazardous waste upon notification from the Wake County District Attorney's Office that the conditions allowing destruction of the blood evidence in North Carolina General Statute § 20-139.1.(h) have been satisfied.

5.2.4.3. If a Motion to Preserve the evidence has been filed and is received by the CCBI Crime Laboratory, the evidence will remain in the custody of the Crime Laboratory or be returned to the submitting agency until dispositive order of a court of competent jurisdiction is entered and received by the Crime Laboratory.

5.3. Evaluate the service requested for the submission, refer to the Administrative Procedure for Review of Requests, Tenders and Contracts for Laboratory Services.

5.3.1. If the request is for GHB or inhalant analysis or is not a DWI submission, issue a RUW notification. If the submission does not contain sufficient blood for analysis issue a RUW notification.

5.4. Record notes which will allow another Blood Drug Chemist to repeat the analysis under conditions as close as possible to the original, evaluate the data, interpret the results, and form an independent conclusion.

5.4.1. Use good laboratory practices at all times to maintain evidence integrity and minimize the risk of cross contamination. Chemists are responsible for laboratory housekeeping which ensures a clean and safe working environment

5.4.1.1. Refer to the Crime Laboratory Health and Safety Manaual and the included Chemical Hygiene Plan.

5.4.1.2. Have only one item of evidence open for analysis at a time.

5.4.1.3. Maintain materials used in analysis that come in direct contact with evidence in closed containers, cabinets or drawers

5.4.1.4. Use disposable glassware, caps and pipette tips for direct contact with evidence, when possible. Properly dispose of such items immediately after use.

5.4.1.5. Label all evidence analysis vessels with identifying information to include the case number.

5.4.1.6. Keep the laboratory work benches and floors free of all items unnecessary for analysis activities at all times.

5.4.1.7. Perform cleaning weekly, at a minimum, to ensure that dust and debris do not accumulate and items unnecessary for analysis are removed. The floors and laboratory work benches should be visibly free from dust and debris at all times.

5.4.1.7.1. Records

5.4.1.7.1.1. Record the weekly cleaning and any comments on the weekly cleaning log form.

5.5. Record all analyses and observations on in the case file as close as possible to the time performed.

5.6. Record the date(s) of examination as "Date started" and "Date completed" on the DWI Blood Chemistry Cover Sheet form. The completion date is the date when all data has been incorporated into a recorded conclusion and the case file is submitted for technical review.

5.7. Record a complete description of the evidence received for each submission.

5.7.1. Include a description of the material, i.e., blood, the tube(s), the color of the stopper(s), all packaging, condition of seals, the location of the subject's name, if present, the subject's name, if present, and submitting agency item numbers.

5.7.2. If a tube appears to contain less than approximately two milliliters of blood or the blood appears abnormal, i.e., leaks, clots, growth, record the observation in the case file.

5.7.3. If multiple tubes are submitted and have recorded differences, clearly identify the tube used for analysis in the case file.

5.7.4. Mark the blood tube(s) with CCBI case number, subject name (if not already present), submitting agency item number (if not already present), initials and date.

5.7.5. If the submission is to be analyzed, only the blood tubes need to be maintained as evidence. Mark any paperwork, other than packaging, submitted with the blood evidence with CCBI case number, initials and date. Store the paperwork in the case file as administrative documentation.

5.7.6. The stopper of the blood tube shall serve as the evidence seal without any additional markings or coverings once the blood tube is received by the Blood Drug Chemist and maintained in their custody.

5.8. Evaluate the evidence received against the submission form. If the subject's name on the evidence is not consistent with the submission form, notify the Quality Manager. If a discrepancy occurs have the DWI Blood Chemistry Technical Leader evaluate the evidence. Record discrepancies in the case file and have the DWI Blood Chemistry Technical Leader verify the discrepancy with initials and date. If necessary, refer to the Administrative Procedure for Review of Requests, Tenders and Contracts for Laboratory Services.

5.8.1. If a major discrepancy occurs, e.g., a missing item of evidence, or tampering is suspected notify the Quality Manager.

5.9. Evaluate the service requested for the submission.

5.9.1. If the DWI submission requests only alcohol analysis or is only specified as "DWI," analyze according to the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography and proceed to reporting.

5.9.2. If the DWI submission contains a request for drug analysis and sufficient blood for both alcohol and drug analysis is present, analyze according to the DWI Blood Chemistry Unit Technical Procedure for Determination of Alcohol and Acetone in Blood by Headspace Gas Chromatography. If sufficient blood is not present for both alcohol and drug analysis, do not perform the alcohol analysis, record the approximate amount of blood present and the decision not to perform the alcohol analysis in the case file and proceed to 5.9.3.

5.9.2.1. If the alcohol analysis is performed and the result is greater than 0.08 gram of alcohol per 100 ml of whole blood and the submission is not indicated to involve a death or serious injury to a victim and additional analysis is not requested by the Wake County District Attorney's Office, do not perform the drug analysis. Proceed to reporting.

5.9.3. If drug analysis is to be performed, analyze according to the DWI Blood Chemistry Unit Technical Procedure for Enzyme Linked Immunosorbent Assay (ELISA) as a Drug Screen.

5.9.3.1. If an indication for barbiturates and / or carisoprodol is observed in the ELISA analysis, then analyze according to the DWI Blood Chemistry Unit Technical Procedure for Solid Phase Extraction of Drugs for GC-MS Analysis.

5.9.3.1.1. Collect and analyze an acidic / neutral fraction and a basic fraction.

5.9.3.1.2. If an indication for benzodiazepines was observed in the ELISA analysis, in addition to the indication for barbiturates and / or carisoprodol, and a benzodiazepine parent compound was not identified in either the acidic / neutral or basic fraction, then also analyze according to the DWI Blood Chemistry Unit Technical Procedure for Solid Phase Extraction of Benzodiazepines for GC-MS Analysis.

5.9.3.2. If only indication(s) for zolpidem, methadone, benzodiazepines, cocaine metabolite, opiates and / or methamphetamine are observed in the ELISA analysis, then analyze according to the DWI Blood Chemistry Unit Technical Procedure for Solid Phase Extraction of Drugs for GC-MS Analysis.

5.9.3.2.1. Collect and analyze a basic fraction. (The acidic/neutral fraction need not be retained or analyzed by GC-MS.)

5.9.3.2.2. If an indication for benzodiazepines was observed in the ELISA analysis, and a benzodiazepine parent compound is not identified, then also analyze according to the DWI Blood Chemistry Unit Technical Procedure for Solid Phase Extraction of Benzodiazepines for GC-MS Analysis.

5.9.3.3. If no indications other than cannabinoids are observed in the ELISA analysis, then analyze according to the DWI Blood Chemistry Unit Technical Procedure for Solid Phase Extraction of Basic Drugs for GC-MS Analysis.

5.9.3.4. If a controlled substance or controlled substance metabolite is indicated by ELISA and identified in a solid phase extraction, refer to 5.10, and either no non-controlled ~~impairing~~ substances / metabolites are identified or analysis for non-controlled ~~impairing~~ substances is not requested by the Wake County District Attorney's Office, then proceed to reporting.

5.9.3.5. If a controlled substance or controlled substance metabolite is identified in a solid phase extraction but is not indicated by ELISA, then repeat the solid phase extraction analysis. If either no non-controlled ~~impairing~~ substances / metabolites are identified

or analysis for non-controlled ~~impairing~~ substances is not requested by the Wake County District Attorney's Office, then proceed to reporting

5.9.3.6. If analysis for non-controlled ~~impairing~~ substances is requested by the Wake County District Attorney's Office and a non-controlled ~~impairing~~ substance / metabolite is identified in a solid phase extraction and is indicated by ELISA, then proceed to reporting. If the non-controlled substance / metabolite is not indicated by ELISA then repeat the solid phase extraction analysis and proceed to reporting.

5.9.3.7. If a positive indication for cannabinoids is observed in the ELISA analysis and no controlled substances or controlled substance metabolites are identified or the cannabinoid confirmation analysis is requested by the Wake County District Attorney's Office, then analyze according to the DWI Blood Chemistry Unit Technical Procedure for THC and THC-COOH for GC-MS Analysis and proceed to reporting.

5.10. Minimum Requirements for Identification and Reporting of Controlled and Non-Controlled Substances

5.10.1. Substance is indicated by ELISA and has a positive full scan mass spectral comparison and has a positive GC RRT comparison

5.10.2. Substance has a positive full scan mass spectral comparison and has a positive GC RRT comparison for each of two sample preparations

5.10.3. Substance has a positive ELISA cannabinoid indication and a positive SIM mass spectral comparison for a cannabinoid and a positive GC retention time comparison for a cannabinoid

5.11. Minimum Requirements for Reporting the Absence of Controlled and Non-Controlled Substances

5.11.1. Non-positive ELISA indications and no analytes identified in a basic solid phase extraction fraction. If an acidic/neutral solid phase extraction fraction is also collected, it must also have no analytes identified.

5.12. Reporting

5.12.1. All DWI submissions analyzed shall be reported on a DWI Blood Chemistry Unit Affidavit and Revocation Report form.

5.12.2. The report shall include a "Description of Evidence Submitted" field. The submitting agency item number(s), a detailed description of the item(s) and seals and, if applicable, the subject name as it appears on the evidence and a description of where it appears on the evidence in this field.

5.12.3. The report shall include a statement of the disposition of the submitted items:

The blood evidence will be retained for a minimum of twelve months from the date this report was notarized. Following that period the blood evidence may be destroyed without further notification according to North Carolina General Statute §20-139.1.(h).

5.12.4. The report shall include a "Results and Conclusions" field. The results and conclusions of the analysis shall be included in this field.

5.12.5. Results and Conclusions Reporting

5.12.5.1. Reporting of alcohol analysis

5.12.5.1.1. When only ethanol is to be reported, report the alcohol concentration and uncertainty with the statement:

The blood alcohol concentration is *(insert blood alcohol concentration*) gram per 100 milliliters ± (*insert uncertainty*) gram per 100 milliliters of whole blood at a coverage probability of 99.73%.

5.12.5.1.2. When multiple alcohols and/or acetone are to be reported, report the concentration and uncertainty with the statement:

The blood alcohol concentration is *(insert blood alcohol concentration*) gram per 100 milliliters ± (*insert uncertainty*) gram per 100 milliliters of whole blood at a coverage probability of 99.73%.
Add for each identified alcohol or acetone:
The (*insert identified alcohol*) concentration is (*insert individual alcohol concentration*) gram per 100 milliliters ± (*insert uncertainty*) gram per 100 milliliters of whole blood at a coverage probability of 99.73%.

5.12.5.1.3. Add an additional statement to the report containing the values from the DWI Blood Chemistry Unit Technical Procedure for Uncertainty of Measurement line 4.13

(The average of the four measured alcohol concentrations is (*insert blood alcohol concentration)* gram per 100 milliliters ± *(insert uncertainty)* gram per 100 milliliters of whole blood at a coverage probability of 99.73%.)

5.12.5.1.4. When compliance with a statutory limit is in question, such as 0.01, 0.04, 0.08 and 0.15 gram of alcohol per 100 milliliters of whole blood, add an additional statement to the report to clearly communicate this information.

Taking the estimated measurement uncertainty into consideration, there is a possibility that the blood alcohol concentration is less than (*insert statutory limit)* gram per 100 milliliters of whole blood.

5.12.5.1.5. If no alcohol is identified or if the alcohol concentration is less than 0.01 grams of alcohol per 100 milliliters of whole blood, report with the statement:

The blood alcohol concentration is 0.00 grams of alcohol per 100 milliliters of whole blood.

5.12.5.2. Reporting of analysis for controlled substances and controlled substance metabolites

5.12.5.2.1. If a positive indication for cannabinoids was observed in the ELISA analysis and the confirmation was not performed report with the statement:

The immunoassay screening test for cannabinoids gave a positive indication for the presence of cannabinoids; however, confirmatory testing was not performed for cannabinoids. ~~Confirmatory testing for cannabinoids will be performed at the request of the Wake County District Attorney's Office.~~

5.12.5.2.2. If a controlled substance or controlled substance metabolite is to be reported, report with the statement:

Analysis for the presence of controlled substances and controlled substance metabolites confirmed the presence of: (*insert the identity of the substance(s)*)

5.12.5.2.3. If no controlled substances or controlled substance metabolites are to be reported, report with the statement:

Analysis for the presence of controlled substances and controlled substance metabolites did not confirm the presence of any controlled substances or controlled substance metabolites.

5.12.5.2.4. If a positive indication, other than cannabinoids, was observed in the ELISA analysis and the presence of a corresponding controlled substance / metabolite was not confirmed, report with the statement:

The immunoassay screening test for (*insert name of immunoassay screening test(s)*) gave a positive indication for the presence of (*insert name of immunoassay screening test(s)*); however, confirmatory testing did not identify drugs indicated by the immunoassay screening test(s).

5.12.5.2.5. If analysis for a specific controlled substance is requested and the substance cannot typically be identified by the DWI Blood Chemistry Unit Technical Procedures, add to the report:

Analysis for (*insert substance(s) requested*) was requested; however, (*insert substance(s) requested*) generally cannot be detected by the current CCBI Crime Laboratory procedures.

5.12.5.2.6. If in the course of analysis for controlled substances / metabolites a non-controlled ~~impairing~~ substance or metabolite was indicated by GC/MS analysis but the criteria for identification and reporting were not met, add the following statement to the report:

Analysis ~~for the presence of non-controlled impairing substances / metabolites~~ indicated the presence of a non-controlled substance. ~~will be completed at the request of the Wake County District Attorney's Office~~.

5.12.5.2.7. If in the course of analysis for controlled substances / metabolites, the identification and reporting requirements for a non-controlled ~~impairing~~ substance / metabolite is met, add to the report:

The presence of the following non-controlled ~~impairing~~ substance(s) / metabolite(s) was confirmed: (*insert the identity of the substance(s)*)

5.12.5.3. Reporting of analysis for non-controlled ~~impairing~~ substances / metabolites

5.12.5.3.1. If a non-controlled ~~impairing~~ substance / metabolite is to be reported, report with the statement:

Analysis for the presence of non-controlled ~~impairing~~ substances / metabolites confirmed the presence of: (*insert the identity of the substance(s)*)

5.12.5.3.2. If no non-controlled ~~impairing~~ substances / metabolites are to be reported, report with the statement:

Analysis for the presence of non-controlled ~~impairing~~ substances / metabolites did not confirm the presence of any non-controlled ~~impairing~~ substances / metabolites.

5.12.5.3.3. If analysis for a specific non-controlled substance is requested and the substance cannot typically be identified by the DWI Blood Chemistry Unit Technical Procedures, add to the report:

Analysis for (*insert substance(s) requested*) was requested; however, (*insert substance(s) requested*) generally cannot be detected by the current CCBI Crime Laboratory procedures.

5.12.6. Additional Reports

5.12.6.1. Amended Reports

5.12.6.1.1. Once a report has been administratively reviewed any alterations to the content of the report may only be done by issuing an amended report.

5.12.6.1.2. In addition to all other report requirements, amended reports must:

5.12.6.1.2.1. Be clearly marked "AMENDED REPORT" in the top left hand corner of the report
5.12.6.1.2.2. Contain the date the amended report was prepared
5.12.6.1.2.3. Contain a reference to the original report
5.12.6.1.2.4. Contain a statement describing the amendment made

5.12.6.2. Supplemental Reports

5.12.6.2.1. Additional analysis results to be reported for evidence which has previously had a CCBI DWI Blood Chemistry Unit Affidavit and Revocation Report issued may only be done by issuing a supplement report.

5.12.6.2.2. In addition to all other report requirements, supplemental reports must:

5.12.6.2.2.1. Be clearly marked "SUPPLEMENTAL REPORT" in the top left hand corner of the report
5.12.6.2.2.2. Contain the date the supplemental report was prepared
5.12.6.2.2.3. Contain a reference to the original report

5.13. Review

5.13.1. All cases shall be subjected to administrative and technical review prior to the release of the report.

5.13.2. The reviews shall be performed in accordance with the CCBI Crime Laboratory Administrative Procedure for Case File Review.

5.13.3. Technical Review

5.13.3.1. The technical review shall be performed by an authorized Blood Drug Chemist other than the analyzing Blood Drug Chemist.

5.13.3.2. The technical review shall include a review of the report and all examination records to ensure:

5.13.3.2.1. Appropriate analyses have been performed and are in conformance with CCBI DWI Blood Chemistry Unit policies and procedures as well as CCBI Crime Laboratory policies and procedures.

5.13.3.2.2. Calculations and data transfers are accurate.

5.13.3.2.3. The conclusions of the analyzing Blood Drug Chemist are reasonable, supported by the examination records and within the constraints of validated scientific knowledge.

5.13.3.2.4. The report is clear, accurate and complete.

5.13.3.3. The Technical Reviewer shall document the review on the DWI Blood Chemistry Coversheet.

5.13.3.3.1. Record any comments in the Review Comments section and initial and date the form.

5.13.3.3.2. If no discrepancies are observed and all requirements in 5.12.3.2. are met, mark the Status as approved. The case is ready for administrative review, refer to 5.12.4.

5.13.3.3.3. If discrepancies are observed and / or all requirements in 5.12.3.2. are not met:

5.13.3.3.3.1. Record all discrepancies in the Review Comments section.

5.13.3.3.3.2. Initial and date the form.

5.13.3.3.3.3. Mark the Status as returned.

5.13.3.3.3.4. Return the case to the analyzing Blood Drug Chemist. Report any significant discrepancies to the Crime Laboratory Deputy Director.

5.13.3.3.3.5. The analyzing Blood Drug Chemist shall make any necessary changes according to the Crime Laboratory Quality Assurance Manual and submit the case for another technical review to the same Technical Reviewer.

5.13.3.3.3.6. If a conflict arises between the analyzing Blood Drug Chemist and the Technical Reviewer that cannot be resolved through discussion and review of procedures and literature, the Blood Drug Chemistry Technical Leader and the Crime Laboratory Deputy Director shall be notified.

5.13.3.3.3.6.1. The Blood Drug Chemistry Technical Leader and the Crime Laboratory Deputy Director shall determine a resolution. The resolution shall be communicated to the analyzing Blood Drug Chemist and the Technical Reviewer and documented in the examination records.

5.13.4. Administrative Review

5.13.4.1. The administrative review shall include:

5.13.4.1.1. A review of the report for spelling and grammatical accuracy.

5.13.4.1.2. A review of all administrative and examination records to ensure that the records are uniquely identified according to CCBI policy and procedure.

5.13.4.1.3. A review of the report to ensure that it is clear, accurate and complete.

5.13.4.1.4. A review of the DWI Blood Chemistry Coversheet to ensure that the technical review has been completed and approved.

5.13.4.2. The administrative review shall be performed by either the Crime Laboratory Deputy Director or a Blood Drug Chemist other than the analyzing Blood Drug Chemist.

5.13.4.3. If no discrepancies are observed and all requirements in 5.12.4.1. are met, mark the Status as approved.

5.13.4.3.1. If discrepancies are observed and / or all requirements in 5.12.4.1. are not met:

5.13.4.3.1.1. Record all discrepancies in the Administrative Review Comments section.

5.13.4.3.1.2. Initial and date the form.

5.13.4.3.1.3. Mark the Status as returned.

5.13.4.3.1.4. Return the case to the analyzing Blood Drug Chemist. Report any significant discrepancies to the Crime Laboratory Deputy Director.

5.14. Upon successful completion of the Administrative and Technical Reviews, sign the Report and have it notarized and disseminated.

6. Limitations

6.1. Refer to the DWI Blood Chemistry Unit technical procedures.

7. Safety

7.1. Refer to the CCBI Crime Laboratory Safety Manual.

8. References

8.1. Levine, Barry ed., *Principles of Forensic Toxicology*. 3rd edition. AACC Press, 2009.

8.2. Moffat, Anthony C. ed. *Clarke's Analysis of Drugs and Poisions,* Volume 1, 4th edition, Pharmaceutical Press, 2011.

8.3. Randall C. Baselt. *Disposition of Toxic Drugs and Chemicals in Man.* 8th Ed. (2008)..

8.4. James C. Garriott (Editor), Medicolegal Aspects of Alcohol, 5th Ed., 2008.

8.5. Baselt, Randall C. *Drug Effects on Psychomotor Performance*, Foster City, California: Biomedical Publications, 2001.

8.6. North Carolina General Statutes.

9. Records

9.1. DWI Blood Chemistry Cover Sheet form
9.2. Weekly Cleaning Log form

Revision History		
Effective Date	Version Number	Reason
2/8/13	1	Compliance with ASCLD/LAB requirements
11/8/13	2	Incorporation of DBCTP14
7/14/14	3	Include use of Agency item numbers and ref to LAPM19
1/16/15	4	Updates, additions, clarifications and minor corrections in 2.3, 5.2, 5.2.1, 5.2.1.1, 5.2.1.2, 5.2.2, 5.2.2.2, 5.2.3, section 5.2.4, section 5.4.1, 5.7.4, 5.7.5, 5.9.3.7, 5.12.3, 5.12.5.1.1, 5.12.5.1.2, 5.12.5.1.3, 5.12.5.1.4, 5.12.5.2.6, 5.12.5.2.7, 9.2, and clarification of Blood Drug Chemist throughout.
8/31/15	5	Update wording for non-controlled substances. Remove wording regarding District Attoney's Office requests for additional analysis from laboratory reports.

14: Technical Procedure for Solid Phase Extraction of Benzodiazepines for GC-MS Analysis

1. **Purpose / Scope -** The procedure is used to isolate benzodiazepine drugs from blood using solid phase extraction for analysis by gas chromatography - mass spectrometry in the DWI Blood Chemistry Unit of the CCBI Crime Laboratory.

2. **Definitions**

   2.1. Quality control check – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

3. **Abbreviations**

   3.1. Refer to DWI Blood Chemistry Unit Technical Procedure for Analysis
   3.2. QCC – Quality control check

4. **Equipment, Materials and Reagents**

   4.1. Equipment

      4.1.1.pH meter
      4.1.2. TurboVap Evaporator
      4.1.3. Mechanical pipettors and corresponding tips
      4.1.4. Volumetric flasks, class A
      4.1.5. Volumetric pipets, class A

   4.2. Materials

      4.2.1. Test tubes, 16 x 125mm and 13 x 100mm
      4.2.2. Test tube caps or stoppers
      4.2.3. Vortex Mixer
      4.2.4. Centrifuge
      4.2.5.UCT Clean Screen DAU Solid Phase Extraction Columns
      4.2.6. Deionized water
      4.2.7. Positive pressure manifold
      4.2.8. Nitrogen, UHP

   4.3. Reagents

      4.3.1. Critical Reagents

4.3.1.1. Negative blood
4.3.1.2. BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)

4.3.2. Reference materials

4.3.2.1. Prazepam

4.3.3. Commercial reagents

4.3.3.1. Ammonium Hydroxide, ACS grade**,**
4.3.3.2. Ethyl Acetate, ACS grade
4.3.3.3. Methanol, ACS grade
4.3.3.4. Acetonitrile, ACS grade
4.3.3.5. Disodium Hydrogen Phosphate, Anhydrous, ACS grade
4.3.3.6. Sodium Dihydrogen Phosphate, Monohydrate, ACS grade

4.3.4. Prepared Reagents

4.3.4.1. 20 % Acetonitrile in 100mM Phosphate Buffer

4.3.4.1.1. Thoroughly mix acetonitrile with 100 mM phosphate buffer to yield the desired volume of a solution containing 20 ~~5~~% acetonitrile. Example: 5 ml acetonitrile and 95 ml 100 mM phosphate buffer.

4.3.4.1.2. Lot number: Eight digit format year/month/day/20%CH3CN:95%Buf/initials of preparer.

4.3.4.1.3. Expiration: end of day

4.3.4.1.4. Storage: closed container

4.3.4.1.5. QCC: successful negative control extraction

4.3.4.2. Ethyl Acetate with 4% Ammonium Hydroxide

4.3.4.2.1. Thoroughly mix ethyl acetate with ammonium hydroxide to yield the desired volume of solution containing 4% ammonium hydroxide. Example: 96 ml ethyl acetate and 4 ml ammonium hydroxide.

4.3.4.2.2. Lot number: Eight digit format year/month/day/AmmEtOAc/initials of preparer.

4.3.4.2.3. Expiration: end of day

4.3.4.2.4. Storage: closed container

4.3.4.2.5. QCC: basic to litmus or pH paper

4.3.4.3. 0.1 M Monobasic sodium phosphate, NaH2PO4

4.3.4.3.1. Dissolve 1.38 ~~1.20~~ grams monobasic sodium phosphate, monohydrate ~~anhydrous~~, in deionized water in a 100 mL volumetric flask.

4.3.4.3.2. Mix and dilute to 100 mL with deionized water.

4.3.4.3.3. Lot number: Eight digit format year/month/day/0.1M_NaH2PO4/initials of preparer

4.3.4.3.4. Expiration: one year

4.3.4.3.5. Storage: closed container

4.3.4.3.6. QCC: Tests acidic with pH or litmus paper

4.3.4.4. 0.1 M Dibasic sodium phosphate, Na2HPO4

4.3.4.4.1. Dissolve 1.42 grams dibasic sodium phosphate, anhydrous, in 80 mL deionized water in a 100 mL volumetric flask.

4.3.4.4.2. Mix and dilute to 100 mL with deionized water.

4.3.4.4.3. Lot number: Eight digit format year/month/day/0.1M_Na2HPO4/initials of preparer.

4.3.4.4.4. Expiration: one year

4.3.4.4.5. Storage: closed container

4.3.4.4.6. QCC: Tests basic with pH paper or litmus paper.

4.3.4.5. 100mM Phosphate Buffer, pH 6.0

4.3.4.5.1. Dissolve 0.85 g Na_2HPO_4 and 6.07 g NaH_2PO_4-H_2O in 400 mL DI water and dilute to 500mL.

4.3.4.5.2. Adjust pH to 6.0 +/- 0.1 with 0.1M monobasic sodium phosphate (lowers

pH) or 0.1M dibasic sodium phosphate (raises pH).

4.3.4.5.3. Lot number: Eight digit format year/month/day/0.1MPhosBuffer/initials of preparer

4.3.4.5.4. Storage: refrigerate in closed container

4.3.4.5.5. Expiration: one month

4.3.4.5.6. QCC: Record final pH

4.3.4.6. Prazepam Internal Standard Stock Solution, 100 µg/ml

4.3.4.6.1. Dilute 1.0 ml of a 1.0 mg/ml prazepam reference standard solution to 10 ml in a class A volumetric flask. Mix.

4.3.4.6.2. Lot number: Eight digit format year/month/day/100µg/mlPrazepamISStock/initials of preparer.

4.3.4.6.3. Storage: freezer in closed container

4.3.4.6.4. Expiration: three years

4.3.4.6.5. QCC: N/A, refer to 4.3.4.7 ~~4.3.4.8~~.

4.3.4.7. Prazepam Internal Standard Solution, 2µg/ml

4.3.4.7.1. Pipet 1.0 ml Prazepam Internal Standard Stock solution, 100 ng/ml, into a 50 ml ~~calss~~ class A volumetric flask. Dilute to volume with methanol.

4.3.4.7.2. Lot number: Eight digit format year/month/day/2µg/mlPrazepamIS /initials of preparer

4.3.4.7.3. Storage: refrigerate in closed container

4.3.4.7.4. Expiration: one year

4.3.4.7.5. QCC: successful negative control extraction

5. Quality Control

5.1. Positive control

5.1.1. Prazepam is added to each basic extraction sample as an internal standard. For each basic

extraction sample, the mass spectrum of the prazepam internal standard must have a positive comparison to prazepam reference material and the signal-to-noise ratio for the prazepam internal standard gas chromatographic peak must be 5 ~~2~~:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.2. Negative Control

5.2.1. For each extraction batch of blood samples prepare a negative control as directed in Section 6 with 2.0 mL of negative blood.

5.2.2. The mass spectrum of the prazepam internal standard of the negative control must have a positive comparison to prazepam reference material and the signal-to-noise ratio for the prazepam internal standard gas chromatographic peak must be 5 ~~2~~:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard ÷ the response at the baseline or valley immediately before the internal standard signal.

5.2.3. The negative control must not contain any controlled substances, controlled substance metabolites or any substance being identified in the sample. The negative control shall be subjected to the same post extraction techniques as any case samples in the batch.

6. Procedure

6.1. Allow all solutions and samples to equilibrate to room temperature.

6.2. Ensure that all blood samples are homogenous by shaking and/or vortexing.

6.2.1. If a homogenous sample cannot be obtained, make a notation in the worksheet detailing the condition of the sample and its handling.

6.3. To 1 mL of 100 mM phosphate buffer add 100 µl of the prazepam internal standard solution, mix/vortex.

6.4. Add 2.0 mL of blood sample to be analyzed (case sample or negative blood for the negative control) using a mechanical pipettor labeled “blood” and add 3 mL of deionized water.

6.5. Mix/vortex and let stand for 5 minutes to lyse red blood cells.

6.6. Mix/vortex sample and centrifuge for 10 minutes at >2000 RPM

6.7. Decant liquid portion of the sample into 2 mL of 100 mM phosphate buffer solution.

6.8. Place the Clean Screen DAU Extraction columns into the positive pressure manifold column rack and place the column rack onto the support arms of the manifold.

6.9. Turn on the nitrogen to the manifold and select the corresponding switches on the front and rear of the manifold to allow nitrogen flow to the corresponding columns.

6.10. Adjust the two pressure regulators on the front of the manifold. Use the low pressure regulator to load or aspirate a sample or liquid from the column at a flow rate of 1 to 2 ml per minute, 1 psi or less. Use the high pressure regulator to dry the column with a pressure setting of 80 psi.

6.11. Condition the column with 3 ml methanol.

6.12. Rinse the column with 3 ml of Ethyl Acetate with 4% Ammonium Hydroxide

6.13. Condition the column with 3 ml deionized water

6.14. Condition the column with 1 ml 100 mM phosphate buffer

6.15. Load sample onto column

6.16. Rinse column with 3 ml deionized water

6.17. Rinse column with 3 ml of 20 ~~5~~% Acetonitrile in 100mM Phosphate Buffer

6.18. Dry column for 5 minutes at 80 psi nitrogen

6.19. Rinse column with 2 ml hexane

6.20. Collect benzodiazepines with 3 ml of Ethyl Acetate with 4% Ammonium Hydroxide

6.21. Evaporate the solvent from the collection test tube using the TurboVap LV Evaporator, refer to the DWI Blood Chemistry Unit Technical Procedure for Biotage TurboVap LV Evaporator. Remove the tubes immediately upon reaching dryness.

6.22. Reconstitute with 50 µl ethyl acetate or perform silylation as directed in 6.22.1 through 6.22.4 for improved analysis by GC-MS.

6.22.1. Derivatize the extract in the collection tube or transfer to a vial using ethyl acetate ~~methanol~~ and evaporate the ethyl acetate ~~methanol~~. Discontinue evaporation immediately upon reaching dryness

6.22.2. Derivatize by adding 50 µl of BSTFA with 1% TMCS to the vial or test tube and capping.

6.22.3. Mix and heat the vial or test tube for 30 minutes at 80°C.

6.22.4. Remove from the heat source and allow the vial or test tube to cool.

6.23. Analyze by GC-MS, refer to the DWI Blood Chemistry Unit Technical Procedure for Gas Chromatography – Mass Spectrometry.

6.24. Record the following in the case file

6.24.1. Lot number of internal standard
6.24.2. Lot number of negative blood
6.24.3. Lot number of UCT Clean Screen DAU Solid Phase Extraction Columns
6.24.4. If applicable, the lot number of BSTFA with 1 % TMCS

7. Limitations

7.1. Refer to the references and other published chemical information as needed to determine the need for derivatization. Some benzodiazepines may need to be derivatized for detection by GC-MS, e.g., a benzodiazepine ELISA indication and no corresponding substance detected in a non-derivatized sample.

7.2. Do not allow the solid phase extraction columns dry during the extraction other than at steps indicated.

7.3. Store solid phase extraction columns in a closed container.

8. Safety

8.1. Refer to the CCBI Crime Laboratory Safety Manual

9. References

9.1. Benzodiazepines in Serum or Plasma for HPLC Analysis Using 200 mg Clean Screen Extraction Column, United Chemical Technologies Inc. Bristol, PA., (2008) 9 –12, 56 – 58.

9.2. BSTFA with 1 % TMCS Product Specification, Sigma-Aldrich Co, (1997).

9.3. Moffat, Anthony C. ed. *Clarke's Analysis of Drugs and Poisions,* Volume 1, 4th edition, Pharmaceutical Press, 2011.

9.4. O'Neal, Maryadele J. ed. The Merck Index – An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co Inc., Whitehouse Station, NJ, (2006).

9.5. Benzodiazepines in Serum or Plasma for HPLC Analysis Using: 200 mg Clean Screen Extraction Column, Internal Publication, United Chemical Technologies Inc. Bristol, PA., (2008).

9.6. Benzodiazepines in Blood Confirmations Using: 200 mg Clean Screen Extraction Column, Internal Publication, United Chemical Technologies Inc. Bristol, PA., (2008).

Revision History		
Effective Date	Version Number	Reason
11/8/13	1	Compliance with ASCLD/LAB requirements
1/16/15	2	Updates and corrections to 4.3.4.1.1, 4.3.4.3.1, 4.3.4.6.5, 4.3.4.7.1, 5.1.1, 5.2.2, 6.17 and 6.22.1.