

Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids

1.0 Purpose - 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 human body fluids or other dilute solutions. This procedure may also be used to qualitatively determine the presence of other volatile compounds human body fluids or dilute solutions.

2.0 Scope – This procedure applies to the Toxicology Units of the State Crime Laboratory.

3.0 Definitions

- **Alcohol** – Any substance containing any form of alcohol, including ethanol, methanol, propanol, and isopropanol (NCGS20-4.0.01).
- **Quality control (QC) check** – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Performance verification** – The initial confirmation of the reliability of a previously or externally validated method or instrument.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Gas chromatograph equipped with flame ionization detectors with Restek BAC1 (Front) and BAC2 (Middle) 30 m x 0.53 mm capillary columns, headspace autosampler and data station.
- 100 µl mechanical pipette
- Hamilton-Microlab 1000 plus/Hamilton 530B diluter/dispenser, or other appropriate liquid handler

4.2 Materials

- Volumetric flasks, Class A: 10, 100, 1000, and 2000 mL (TC) sizes
- Volumetric pipettes, Class A: 1, 5, 8, 10, 20, and 50 mL (TD) sizes
- Headspace vials with sealing caps
- Crimper tool

4.3 Reagents

- Deionized water
- Sodium chloride

4.4 Commercial Reagents

- Helium gas
- Hydrogen gas
- Nitrogen gas
- Compressed air

4.5 Primary Reference Materials

- Ethanol, 200 proof, ACS Grade
- n-Propanol, min. 99.5 %
- Isopropanol, USP Grade
- Acetone, ACS Grade
- Methanol, ACS Grade
- NIST traceable multi-component alcohol Certified Reference Material solutions containing ethanol, methanol, acetone and isopropanol

4.6 Critical Reagents

- Multi-component Alcohol Certified Reference Material solutions (ethanol, methanol, acetone and isopropanol), NIST traceable, containing 0.050 g/100 mL, 0.100 g/100mL and 0.400 g/100 mL of each component.

4.7 Prepared Reagents - The reagents may be prepared by a qualified Forensic Scientist or Chemistry Technician in any amount provided that the component ratios are kept constant.

4.7.1 BAC (Blood Alcohol Concentration) Stock Calibration Solution

4.7.1.1 Prepare a 1.00 gram/100 mL solution of primary reference material ethanol, primary reference material methanol, primary reference material acetone, and primary reference material isopropanol. Record the weight of each component.

4.7.1.1.1 Example: Weigh 10.0 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.

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

4.7.1.2.1 Example: 20101231BacStockCalXXX

4.7.1.3 Expiration: One year.

4.7.1.4 Refrigerate.

4.7.1.5 QC Check: Not applicable (see BAC Working Calibration Solutions).

4.7.2 BAC Stock Verification Solution

4.7.2.1 Prepare a 1.00 gram/100 mL solution of primary reference material ethanol, primary reference material methanol, primary reference material acetone, and primary reference material isopropanol. Record the weight of each component.

4.7.2.1.1 Example: Weigh 10.0 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.

4.7.2.2 Lot Number: Eight digit format year/month/day/BacStockVer/initials of preparer.

4.7.2.2.1 Example: 20101231BacStockVerXXX

4.7.2.3 Expiration: One year.

4.7.2.4 Refrigerate.

4.7.2.5 QC Check: Not applicable (see BAC Working Verification Solutions).

4.7.3 BAC Working Calibration Solutions

4.7.3.1 Prepare 0.010, 0.040, 0.080, 0.200, and 0.500 gram/100 mL calibration solutions from the BAC Stock Calibration Solution using the appropriate Class A pipette for each solution.

4.7.3.1.1 Example: Pipette 1, 4, 8, 20, and 50 mL respectively 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.

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

4.7.3.2.1 Example: 20101231Bac0.010CalAAA

4.7.3.3 Expiration: Three months.

4.7.3.4 Refrigerate.

4.7.3.5 QC Check: Successful calibration (refer to **5.4**).

4.7.4 BAC Working Verification Solution

4.7.4.1 Prepare 0.015 gram/100 mL verification solution from the BAC Stock Verification Solution using the appropriate Class A pipette.

4.7.4.1.1 Example: Pipette 1.5 mL of the stock verification solution into separate 100 mL volumetric flask containing approximately 50 mL deionized water. Bring the flask to volume with deionized water.

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

4.7.4.2.1 Example: 20101231Bac0.015VERAAA

4.7.4.3 Expiration: Three months.

4.7.4.4 Refrigerate.

4.7.4.5 QC Check: Successful calibration (refer to **5.4**).

4.7.5 BAC Internal Standard (n-propanol)

4.7.5.1 Prepare a 0.050 gram/100 mL of primary reference standard n-propanol.

4.7.5.1.1 Example: weigh 1.0 gram of n-propanol into a beaker. Quantitatively transfer to a 2000 mL volumetric flask. Bring the flask to volume with deionized water.

4.7.5.2 Add 10 g sodium chloride per 1 liter of internal standard produced to make a 1 % solution of sodium chloride.

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

4.7.5.3.1 Example: 20101231BacISXXX

4.7.5.4 Expiration: Three months.

4.7.5.5 Store tightly closed at room temperature.

4.7.5.6 QC Check: Successful calibration (refer to **5.4**).

5.0 Procedure

5.1 Instrument Performance Verification for New Instrumentation

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

5.1.2 Performance verification shall be performed on new gas chromatographs prior to being used for casework.

5.1.3 Performance verification shall include successful calibration (refer to **5.4**), daily system check and analysis of a minimum of fifteen blood samples with known results. All quality control requirements shall be met.

5.1.3.1 The known blood samples may be prepared or purchased.

5.1.3.2 The results of the known samples shall be compared to their known values and shall be within +/- 5.0 percent.

5.1.4 The data shall be filed and maintained by the Blood Alcohol Key Operator to document the new instrument set up.

5.1.5 A new entry for the instrument shall be made in the Resource Manager section of FA prior to use in casework. The new entry shall include the following:

- Manufacturer's serial number.
- Unique section identifier for the new instrument.
- Notation under "Verification Date" to reflect the date the performance verification was completed.

5.2 Maintenance

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

5.2.2 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.

5.2.3 The Blood Alcohol Key Operator or designee shall update the instrument log when the instrument is ready to be used for casework and file any generated data in the instrument notebook located near the instrument

5.2.4 Suggested Routine Maintenance Schedule

5.2.4.1 This is a suggested maintenance schedule. Instrument use may alter the need for maintenance. The maintenance schedule shall be determined by the Blood Alcohol Key Operator or designee based upon instrument use.

5.2.4.2 Septum

5.2.4.2.1 Replace weekly when in use.

5.2.4.2.2 A successful daily system check (see **5.5.1**) shall be performed prior to analyzing samples.

5.2.4.3 Syringe

5.2.4.3.1 Replace every six months.

5.2.4.3.2 A successful daily system check (see **5.5.1**) shall be performed prior to analyzing samples.

5.2.4.4 Liner

5.2.4.4.1 Replace every twelve months.

5.2.4.4.2 A successful daily system check (see **5.5.1**) shall be performed prior to analyzing samples.

5.2.4.5 Jet

5.2.4.5.1 Inspect every twelve months, replace as needed.

- 5.4.4** The calibration curve shall be fitted to a linear model.
- 5.4.5** The calibration curves for each component shall show a correlation of determination (r^2) of 0.995 or greater. If the calibration has a correlation of determination of less than 0.995, appropriate action (i.e., maintenance or new solution preparation) shall be taken and the calibration repeated.
- 5.4.6** After each successful calibration, save the data analysis method according to the format “BacGC” instrument number and eight digit format year/month/day.
- 5.4.7 Calibration Verification**
- 5.4.7.1** Prepare each of the commercial multi-component alcohol certified reference material solutions (0.050 g/100 mL, 0.100 g/100 mL and 0.400 g/100mL) in triplicate according to **5.6**.
- 5.4.7.2** Prepare the BAC working verification solution of 0.015 g/100mL in triplicate according to **5.6**.
- 5.4.7.3** Analyze the verification samples on the gas chromatograph.
- 5.4.7.4** Quantitate the samples with the updated calibration.
- 5.4.7.5** Each component shall be identified by the instrument software on both columns and be visually baseline resolved.
- 5.4.7.6** All samples with target concentrations greater than 0.08 g/100 mL, evaluate each component’s quantitation results.
- 5.4.7.6.1** All components’ quantification results shall be within +/- 5.0 % of the target. Any components other than ethanol that do not meet this requirement shall not be quantitated.
- 5.4.7.7** All samples with target concentrations less than 0.08 g/100 mL, evaluate each component’s quantitation results.
- 5.4.7.7.1** All components’ quantification results shall be within +/- 0.004 g/100 mL of the target. Any components other than ethanol that do not meet this requirement shall not be quantitated.
- 5.4.8** Calibrations shall meet all requirements for each component to be quantitated in casework. If a component does not meet all requirements it shall be noted in the instrument log and FA.
- 5.4.9** If the calibration is found to be unacceptable for ethanol, appropriate action (e.g., maintenance or new solution preparation) shall be taken and the calibration repeated.
- 5.4.10** Calibration data shall be reviewed by another a Forensic Scientist qualified to perform [Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids](#) and, if acceptable, approved in the Toxicology Unit section object repository in FA with a file

name beginning with "BacCal" (capitalization optional) and the date in yyyyymmdd format with no space between them followed by the instrument number(s).

5.4.10.1 Examples: BacCal20080101GC1 or BacCal20080101GC1and2.

5.4.11 Calibration data shall include the lot numbers and expiration dates of the calibration standards, commercial and working standards and internal standard; the chromatograms for each calibration and verification standard; a copy of the instrumental method; the printed calibration table; and the r^2 values of each component.

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

5.4.12.1 Record any components that do not meet the requirements.

5.5 Standards and Controls

5.5.1 Daily System Check

5.5.1.1 The daily system check shall be performed in the twenty-four hours preceding any sample analysis. The daily system check is valid for twenty four hours after the injection of the first verification standard.

5.5.1.2 Prepare each of the multi-component alcohol certified reference material solutions (0.050 g/100 mL, 0.100 g/100 mL and 0.400 g/100mL) according to **5.6**.

5.5.1.3 Chromatograph the prepared samples in a single sequence with the negative control after the 0.400 g/100 mL multi-component alcohol certified standard verification sample.

5.5.1.4 Analyze the samples with the current data analysis method corresponding to the internal standard used.

5.5.1.4.1 Each verification sample component shall be identified by the instrument software on both columns and be visually baseline resolved.

5.5.1.4.2 For the duplicates of each verification sample with target concentrations greater than 0.08 g/100 mL, evaluate each component's quantitation results.

5.5.1.4.2.1 All components' quantitation results shall be within +/- 5.0 % of the target. Any components that do not meet this requirement shall not be quantified.

5.5.1.4.3 For the 0.05 g/100 mL verification sample duplicates, evaluate each component's quantitation results.

5.5.1.4.3.1 All components' quantitation results shall be within +/- 0.004 g/100 mL of the target. Any components that do not meet this requirement shall not be quantified.

5.5.1.4.4 The negative control shall not contain any identifiable methanol, ethanol, isopropanol, acetone or any other identifiable volatile.

5.5.1.5 A daily system check shall meet all requirements for each component to be quantitated in casework. If a component does not meet all requirements it shall be noted in the instrument log and FA.

5.5.1.6 If the daily system check is found to be unacceptable for ethanol, appropriate action (e.g., maintenance or new solution preparation) shall be taken and the daily system check repeated in accordance with the State Crime Laboratory [Procedure for Corrective Action and Non-Conformities](#).

5.5.1.7 The daily system check data shall be reviewed by a Forensic Scientist qualified to perform [Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids](#) and, if acceptable, approved in the Toxicology Unit section object repository of FA with a file name beginning with "BacDsc" (capitalization optional) followed by eight digit format year/month/day and the instrument number(s).

5.5.1.7.1 Examples: BacDsc20080818GC1 or
BacDsc20080101GC1and2.

5.5.1.8 The daily system check data shall include the sequence table, the chromatograms for each verification sample and negative control, the lot numbers of the multi-component certified reference material solutions and the lot number of the internal standard solution.

5.5.1.9 Control Charting

5.5.1.9.1 Complete the Toxicology Control Chart Form and submit to the Toxicology Technical Leader.

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

5.5.1.10.1 Record any components that do not meet the requirements.

5.5.2 Quality Control for body fluids

5.5.2.1 A sample sequence shall contain a minimum of 10 % control samples.

5.5.2.2 The first and last samples of a sequence shall be a positive and negative control sample with any remaining required control samples distributed throughout the batch.

- 5.5.2.3** The target concentration of the positive controls shall be selected to avoid duplicate concentrations within a sequence when possible.
- 5.5.2.4 Negative control**
- 5.5.2.4.1** Prepare a water sample according to **5.6**.
- 5.5.2.4.2** Negative controls shall not contain any identifiable methanol, ethanol, isopropanol, acetone or any other identifiable volatile.
- 5.5.2.5 Positive control**
- 5.5.2.5.1** Prepare the number and concentrations needed of the Commercial Multi-component Alcohol Certified Reference Material Solutions (0.050 g/100 mL, 0.100 g/100 mL or 0.400 g/100mL) according to **5.6**.
- 5.5.2.5.2** For each positive control with a target concentration greater than 0.08 g/100 mL, evaluate each component's quantitation results.
- 5.5.2.5.2.1** All components' quantification results shall be within +/- 5.0 % of the target. Any components that do not meet this requirement shall not be quantitated
- 5.5.2.5.3** For each positive control with a target concentration of 0.08 g / 100 mL or less, evaluate each component's quantitation results.
- 5.5.2.5.3.1** All components' quantification results shall be within +/- 0.004 g/100 mL of the target. Any components that do not meet this requirement shall not be quantitated.
- 5.5.2.6** Sample sequence quality control samples shall meet all requirements for each component to be quantitated in casework. If a component does not meet all requirements it shall be noted in the instrument log and FA.
- 5.5.2.7** If a quality control sample in a sequence is found to be unacceptable for a component, all cases after the last acceptable quality control sample that contain an integrated peak for that component in all four chromatograms shall be reanalyzed. This shall be documented in the case file. The data may be used for the purpose of identification only.
- 5.5.2.7.1** Record the unacceptable quality control sample in the instrument log and FA.
- 5.5.2.7.2** Notify the Blood Alcohol Key Operator or designee.

5.5.2.7.3 Correct any apparent problem and document the action in the instrument log.

5.5.2.7.4 Successful daily system check (**5.5.1**) shall be performed prior to resuming casework.

5.5.2.8 The Quality Control data shall be reviewed by a Forensic Scientist qualified to perform [Headspace Gas Chromatography to Quantitate and Identify Volatiles in Liquids](#) and, if acceptable, approved in the Toxicology Unit section object repository of FA with a file name beginning with “BacQc” (capitalization optional), followed by eight digit format year/month/day and the instrument number(s). A suffix shall be added to the name of the file to distinguish between multiple runs on a single day.

5.5.2.8.1 Examples: BacQc20080818GC1-XXX or
BacQc20080101GC1and2-XXX.

5.5.2.9 The Quality Control data shall include the sequence table, the chromatograms for each control sample, the lot numbers of the multi-component certified reference material solutions, and internal standard solution

5.5.2.10 Record each sequence in the instrument log with the date and time of the first injection and operator initials.

5.5.2.11 Control Charting

5.5.2.11.1 Complete the Toxicology Control Chart Form and submit to the Toxicology Technical Leader.

5.5.3 Quality Control for Alcoholic Beverage Analysis

5.5.3.1 The requirements are the same as those under **5.5.2**. Add a dilute ethanol positive control.

5.5.3.2 Dilute ethanol positive control

5.5.3.2.1 Prepare a 50 % (by volume) ethanol solution by pipetting 5.0 mL of ethanol certified standard into a 10 mL volumetric flask with a Class A pipette and diluting to volume with water. Other concentrations may be prepared if needed. If acetone or an alcohol other than ethanol is to be identified and quantified, a dilute positive control containing that component shall be prepared from a reference material.

5.5.3.2.2 For each positive control, pipette 100 µL of the prepared solution into a 10 mL volumetric flask and dilute to volume with deionized water in duplicate.

5.5.3.2.2.1 Record the pipette used to make this control in the case record

5.5.3.2.3 Prepare each singly according to **5.6.1** thru **5.6.4**.

5.5.3.2.4 Set the multiplier in the sequence table to 1267.

5.5.3.2.4.1 Calculation to obtain the 1267 value - multiply the average g/100 mL result by 100 to compensate for the original dilution, multiply by 10 to compensate for the dilution with internal standard and divide by 0.7893 (density of ethanol) to convert from g/mL to mL/mL.

5.5.3.2.5 Average the four measured values and truncate to the tenths place to obtain the measured percent by volume of ethanol.

5.5.3.2.6 Any values that exhibit greater than 5 % difference as described by the equation; $[(\text{highest measured percent by volume} - \text{lowest measured percent by volume}) / \text{lowest measured percent by volume}] \times 100$ shall not be quantitated.

5.5.3.2.7 The average of each component's quantitation results shall be within +/- 5.0 % of the target. Any components that do not meet this requirement shall not be quantitated.

5.6 Alcohol and Acetone Concentration in body fluids

5.6.1 Sampling

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

5.6.1.2 Ensure that all body fluids are homogenous by shaking and/or vortexing.

5.6.1.2.1 If a homogenous sample cannot be obtained due to the presence of clots, a notation shall be made in the FA worksheet and the alcohol concentration shall be calculated according to **5.6.3.6**.

5.6.1.2.2 If a homogenous sample cannot be obtained because the blood cells have been separated from the liquid, a notation shall be made in the FA worksheet and the alcohol concentration shall be calculated according to **5.6.3.6**.

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

5.6.1.3 With an appropriate liquid handler, deliver 1.80 mL of the BAC Internal Standard Solution and 0.20 mL of the liquid to be analyzed into a headspace vial labeled with the appropriate identifying information and cap securely.

5.6.1.4 Prepare each liquid to be analyzed in replicate and chromatograph using a BAC method.

5.6.1.4.1 The replicate set shall have the case samples pipetted and analyzed in reverse order from the first set.

5.6.1.5 Volatiles that elute after the end of the run time defined in **5.3** are considered late eluting volatiles. If late eluting volatiles are requested or indicated, the case shall be re-analyzed in replicate using the Volramp method. The Volramp method is a BAC method with a modified run time and temperature ramp. Quantitation shall not be performed with the Volramp method.

5.6.1.6 Analyze each sample using the most current BAC method corresponding to the instrument used and the lot of BAC Internal Standard Solution used in sample preparation.

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

5.6.2 Identification of volatiles

5.6.2.1 Ethanol, methanol, isopropanol or acetone shall be integrated in the appropriate retention time window on both columns in both sample preparations to be identified. Refer to **5.3.4** for retention time window settings.

5.6.2.2 Include the sample chromatograms, calibration data, daily system check data and quality control data in the case record.

5.6.2.3 Volatiles other than those listed in **5.6.2.1** may be identified using a reference material relative retention time (RRT) comparison relative to the internal standard.

5.6.2.3.1 The gas chromatographic RRT of the sample and reference material shall not differ by more than 2.0 % on each column in both sample preparations.

5.6.2.3.2 The reference material shall be analyzed under the same chromatographic conditions as the sample.

5.6.2.3.3 Include the sample chromatograms, the sample RRT, the reference material RRT, calibration data, daily system check data and quality control data in the case record.

5.6.2.4 Volatiles other than those listed in **5.6.2.1** may be identified using a reference material retention time comparison.

5.6.2.4.1 The gas chromatographic retention time of the sample and reference material shall not differ by more than 2.0 %.

5.6.2.4.2 The reference material shall be analyzed under the same chromatographic conditions within thirty days of the sample.

5.6.2.4.3 Include the sample and reference material chromatograms with the retention times displayed, the calibration data, daily system check data and quality control data in the case record.

5.6.2.5 Reporting

5.6.2.5.1 Refer to the Toxicology Unit [Toxicology Analysis](#) procedure for reporting of identified alcohols, acetone and other volatiles.

5.6.3 Determination of alcohol or acetone concentration

5.6.3.1 The concentrations of ethanol, methanol, isopropanol and acetone shall be measured and calculated to the ten thousandths place by the instrument software utilizing the most current calibration that corresponds to the instrument used and the lot of BAC Internal Standard solution used to prepare the samples. The mean of the four measured values obtained for each component is the concentration of that component. If any of the four measured values for a component are below the lowest calibrator of 0.01 g/100mL, the component shall be reported as negative.

5.6.3.2 The Blood Alcohol Concentration (BAC) 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.

5.6.3.3 The concentration of acetone in blood and the concentrations of ethanol, methanol, isopropanol and acetone in other body fluids shall be truncated to the hundredths place.

5.6.3.4 Any identified alcohol or acetone values that exhibit greater than 5 % difference as described by the equation: $[(\text{highest measured concentration} - \text{lowest measured concentration}) / \text{lowest measured concentration}] \times 100$, shall be rerun.

5.6.3.5 Samples where the highest measured concentration is 0.080 grams or less per 100 milliliters need not be rerun if the measured concentrations are not separated by greater than 0.004 grams per 100 milliliters.

5.6.3.6 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 1.18 to compensate for the whole blood:serum alcohol distribution ratio.

5.6.3.7 Reporting

5.6.3.7.1 Refer to the Toxicology Unit Technical Procedure for Toxicology Analysis.

5.6.4 Calculations

- 5.6.4.1** Alcohol or acetone values percent difference: $[(\text{highest measured concentration} - \text{lowest measured concentration}) / \text{lowest measured concentration}] \times 100$
- 5.6.4.2** Relative retention time (RRT), round to the nearest hundredth: $(\text{analyte retention time} / \text{internal standard retention time})$
- 5.6.4.3** Relative retention time (RRT) percent difference calculation, round to one decimal place: $[(\text{standard relative retention time} - \text{analyte relative retention time}) / (\text{standard relative retention time})] \times 100$
- 5.6.4.4** 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 1.18.

5.6.5 Uncertainty of Measurement – N/A

5.7 Alcoholic beverage analysis

5.7.1 Sampling

- 5.7.1.1** Allow all solutions and samples to be analyzed to equilibrate to room temperature.
- 5.7.1.2** Ensure that all solutions are homogenous by shaking and/or vortexing.

5.7.2 Pipette 100 μL of the liquid to be analyzed into a 10 mL volumetric flask and dilute to volume with deionized water in duplicate.

5.7.2.1 Record the pipette used to make the dilution in the case record.

5.7.3 With an appropriate liquid handler, deliver 1.80 mL of the BAC Internal Standard Solution and 0.20 mL of the dilution to be analyzed into a headspace vial and cap securely.

5.7.4 Chromatograph using a BAC method.

5.7.4.1 Volatiles that elute after the end of the run time defined in **5.3** are considered late eluting volatiles. If late eluting volatiles are requested or indicated, the case shall be re-analyzed in replicate using the Volramp method. The Volramp method is a BAC method with a modified run time and temperature ramp. Quantitation shall not be performed with the Volramp method.

5.7.5 Analyze each sample using the most current BAC method corresponding to the instrument used and the lot of BAC Internal Standard Solution used in sample preparation.

5.7.6 Set the multiplier in the sequence table to 10.

5.7.7 Identification of volatiles

5.7.7.1 Refer to **5.6.2**.

5.7.8 Determination of alcohol or acetone concentration

5.7.8.1 The concentrations of ethanol, methanol, isopropanol and acetone shall be measured and calculated to the ten thousandths place by the instrument software utilizing the most current calibration data that corresponds to the instrument used and the lot of internal standard solution used to prepare the samples.

5.7.8.2 If the sample is positive for ethanol, multiply the ethanol average g/100 mL result by 126.7 and round to the tenths place to obtain the percent by volume of that component in the original sample.

5.7.8.3 If the sample is positive for methanol, multiply the methanol average g/100 mL result by 126.3 and round to the tenths place to obtain the percent by volume of that component in the original sample.

5.7.8.4 If the sample is positive for isopropanol, multiply the isopropanol average g/100 mL result by 127.4 and round to the tenths place to obtain the percent by volume of that component in the original sample.

5.7.8.5 If the sample is positive for acetone, multiply the acetone average g/100 mL result by 126.3 and round to the tenths place to obtain the percent by volume of that component in the original sample.

5.7.8.6 Any identified alcohol or acetone values that exhibit greater than 5 % difference as described by the equation; $[(\{\text{highest measured percent by volume minus lowest measured percent by volume}\} \div \text{lowest measured percent by volume}) \times 100]$, shall be rerun.

5.7.8.7 Reporting

5.7.8.7.1 Refer to the Toxicology Unit [Toxicology Analysis](#) procedure.

5.7.9 Calculations

5.7.9.1 Alcohol or acetone values percent difference: $[(\{\text{highest measured concentration minus lowest measured concentration}\} \div \text{lowest measured concentration}) \times 100]$

5.7.9.2 The multiplier value is obtained by multiplying the average g/100 mL result by 100 to compensate for the original dilution, multiplying by 10 to compensate for the dilution with internal standard and dividing by the density of the appropriate analyte to convert from g/mL to mL/mL.

5.7.10 Uncertainty of Measurement – N/A

6.0 Limitations

- 6.1 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 1.18 to compensate for the whole blood:serum (plasma) alcohol distribution ratio.

7.0 Safety

- 7.1 Refer to the State Crime Laboratory Safety Manual.

8.0 References

James C. Garriott. *Medicolegal Aspects of Alcohol*. 3rd Ed. (1996).

James C. Garriott . *Medicolegal Aspects of Alcohol*. 5th Ed. (2008).

Operation Manual(s) for the gas chromatograph.

Operation Manual(s) for the headspace autosampler.

Operation Manual(s) for the data system and applicable software.

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

Macchia T., et al. "Ethanol in Biological Fluids: Headspace GC Measurement." *Journal of Analytical Toxicology*. 1995, Vol. 19, 4, (Jul-Aug): 241-6.

Weast, Robert C. *CRC Handbook of Chemistry and Physics*. 66th Ed. (1985).

9.0 Records

- Case record
- Quality control data
- Daily system check data
- Calibration data
- GC Instrument log
- Toxicology Control Chart Form
- GC Maintenance log

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	J-02 and J-11 combination and conversion to ISO format
10/26/2012	2	5.3.4 - removed "after maintenance"; 5.5.1.2 - removed "in duplicate"; 5.5.1.3, 5.5.1.4.4, and 5.5.1.8 - changed "water blank to "negative control"; inserted 5.5.1.9 - changed language to reflect new control chart form; 5.5.2.2 - removed "evenly"; 5.5.2.8 - inserted language for the addition of a suffix; 5.5.2.11 and 5.5.2.12 - combined to reflect new Toxicology Control Chart Form; 5.6.1.4.1 - inserted reverse order criteria; 5.6.1.5 and 5.7.4.1 - clarified and reworded due to change in 5.6.1.4.1; 9.0 - removed old control charts and inserted consolidated Toxicology Control Chart Form; grammar
02/15/2013	3	2.0 - modified for procedure merge 5.3 - removed sections 5.3.1-5.3.3, made 5.3.4 new 5.3