

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 bodily fluids or other dilute solutions. This procedure may also be used to qualitatively determine the presence of other volatile compounds in bodily 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).
- **Alcoholic beverage** – “any beverage containing at least one-half of one percent (0.5 %) alcohol by volume, including malt beverages, unfortified wine, fortified wine, spirituous liquor, and mixed beverages.” (NCGS 18B-101 (4))
- **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), or equivalent 30 m x 0.53 mm capillary columns, headspace auto-sampler 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
- Calibrated mechanical pipettes
- 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.

4.7.1.1.1 Weigh 10.0 g each of ethanol, methanol, acetone, and isopropanol into a beaker.

4.7.1.1.2 Quantitatively transfer each into a single 1000 mL volumetric flask.

4.7.1.1.3 Bring the flask to volume with deionized water.

4.7.1.1.4 Amounts may be increased or decreased proportionally to produce a different final solution volume.

4.7.1.2 Lot Number: Eight digit format year/month/day

4.7.1.2.1 Example: 20101231

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.

4.7.2.1.1 Weigh 10.0 g each of ethanol, methanol, acetone, and isopropanol into a beaker.

4.7.2.1.2 Quantitatively transfer each into a single 1000 mL volumetric flask.

4.7.2.1.3 Bring the flask to volume with deionized water.

4.7.2.1.4 Amounts may be increased or decreased proportionally to produce a different final solution volume.

4.7.2.2 Lot Number: Eight digit format year/month/day

4.7.2.2.1 Example: 20101231

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 pipette for each solution.

4.7.3.1.1 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.

4.7.3.1.2 Bring each flask to volume with deionized water.

4.7.3.1.3 Amounts may be increased or decreased proportionally to produce a different final solution volume.

4.7.3.2 Lot Numbers: Eight digit format year/month/day

4.7.3.2.1 Example: 20101231

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 pipette.

4.7.4.1.1 Pipette 1.5 mL of the stock verification solution into separate 100 mL volumetric flask containing approximately 50 mL deionized water.

4.7.4.1.2 Bring the flask to volume with deionized water.

4.7.4.1.3 Amounts may be increased or decreased proportionally to produce a different final solution volume.

4.7.4.2 Lot Numbers: Eight digit format year/month/day

4.7.4.2.1 Example: 20101231

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 Weigh 1.0 gram of n-propanol into a beaker.

4.7.5.1.2 Quantitatively transfer to a 2000 mL volumetric flask.

4.7.5.1.3 Bring the flask to volume with deionized water.

4.7.5.1.4 Amounts may be increased or decreased proportionally to produce a different final solution volume.

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
 - 4.7.5.3.1** Example: 20101231
- 4.7.5.4** Expiration: Six months.
- 4.7.5.5** Store tightly closed at room temperature.
- 4.7.5.6** QC Check:
 - 4.7.5.6.1** Prepare a Negative Control (refer to **5.4.2.4.1**) using new lot of internal standard.
 - 4.7.5.6.2** Prepare a Negative Control (refer to **5.4.2.4.1**) using previous lot of internal standard.
 - 4.7.5.6.3** Analyze both Negative Controls using current calibration method on all gas chromatographs to be calibrated. The results from the analysis must meet criteria in **5.4.2.4.2**.
 - 4.7.5.6.4** Place QC check data files in FA. The data must be approved by the Toxicology Technical Leader or designee prior to calibration.

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 the following: successful calibration (refer to **5.4**), daily system check and analysis of a minimum of ten 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 When maintenance is performed, the peak retention times shall be updated to the retention times of the 0.100 g/100 mL positive control from the Daily System Check.

5.2.4 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

5.2.5 Suggested Routine Maintenance Schedule

5.2.5.1 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.

5.2.5.2 Septum

5.2.5.2.1 Replace weekly when in use.

5.2.5.2.1.1 A successful daily system check (see **5.4.1**) shall be performed prior to analyzing samples.

5.2.5.3 Syringe

5.2.5.3.1 Inspect every twelve months, and replace as needed.

5.2.5.3.1.1 A successful daily system check (see **5.4.1**) shall be performed prior to analyzing samples.

5.2.5.4 Liner

5.2.5.4.1 Inspect every twelve months, and replace as needed.

5.2.5.4.1.1 A successful daily system check (see **5.4.1**) shall be performed prior to analyzing samples.

5.2.5.5 Jet

5.2.5.5.1 Inspect every twelve months, and replace as needed.

5.2.5.5.1.1 A successful daily system check (see **5.4.1**) shall be performed prior to analyzing samples.

5.2.5.6 Column

5.2.5.6.1 Bake column prior to calibration and as needed.

5.2.5.6.1.1 A successful daily system check (see 5.4.1) shall be performed prior to analyzing samples.

5.2.5.6.2 Replace as needed based upon quality of resolution obtained in the daily system check.

5.2.5.6.2.1 A successful calibration and daily system check shall be performed prior to analyzing samples.

5.2.6 Non-routine Maintenance

5.2.6.1 All non-routine maintenance shall, at a minimum, be followed by a successful daily system check (see **5.4.1**) prior to analyzing samples.

5.2.6.2 Non-routine maintenance shall be evaluated by the Blood Alcohol Key Operator or designee to determine the need for recalibration prior to analyzing samples.

5.2.7 Reboot

5.2.7.1 Successful daily system check (see **5.4.1**) shall be performed following GC or auto-sampler reboot.

5.3 Calibration

5.3.1 The Blood Alcohol Key Operator or designee shall calibrate the GC upon preparation of a new lot of Internal Standard solution and after instrument maintenance that may affect the calibration.

5.3.2 Prepare each of the calibration solutions from **4.7.3** in triplicate according to **5.5**.

5.3.3 Using the instrument software, update the calibration table with the retention times and responses of the calibration samples.

5.3.3.1 The response at each concentration shall be determined by the average response of the triplicates analyzed at that concentration.

- 5.3.3.2** The retention time for each component shall be that of the average of all calibration samples analyzed.
- 5.3.3.2.1** The peak retention time windows shall be set to the peak retention time +/- (0.05 minutes +0.5 % of the peak retention time).
- 5.3.4** The calibration curve shall be fitted to a linear model.
- 5.3.5** 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.
- 5.3.6** After each successful calibration, save the data analysis method according to the format "BacGC" instrument number and eight digit format year/month/day.
- 5.3.7 Calibration Verification**
- 5.3.7.1** 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 **5.5**.
- 5.3.7.2** Prepare the BAC working verification solution of 0.015 g/100mL in triplicate according to **5.5**.
- 5.3.7.3** Analyze the verification samples on the gas chromatograph.
- 5.3.7.4** Quantitate the samples with the updated calibration.
- 5.3.7.5** Each component shall be identified by the instrument software on both columns and be visually baseline resolved.
- 5.3.7.6** All ethanol quantitation results shall be within +/- 5.0 % of the target.
- 5.3.7.7** All Methanol, isopropanol, and acetone quantitation results shall be within +/- 10 % of the target.
- 5.3.8** 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 on the calibration file (refer to 5.3.10).
- 5.3.9** 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.
- 5.3.10** Calibration 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 in FA with a file name beginning with "BacCal" (capitalization optional) and the date in yyymmdd format with no space between them followed by the instrument number(s).

5.3.10.1 Examples: BacCal20080101GC1 or BacCal20080101GC1and2.

5.3.11 Calibration data shall include the appropriate FA workstation (refer to the [Toxicology Quality Assurance Procedure](#)); the Calibration sequence table(s); 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.

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

5.3.12.1 Record any components that do not meet the requirements.

5.4 Standards and Controls

5.4.1 Daily System Check

5.4.1.1 The daily system check shall be performed on the day of analysis and preceding any sample analysis. The daily system check is valid for twenty four hours after the injection of the first verification standard.

5.4.1.2 Prepare a 0.100 g/100 mL multi-component alcohol certified reference material solution and a negative control according to **5.5**.

5.4.1.2.1 The replicate analysis required in **5.5.1.4** does not apply to the Daily System Check.

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

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

5.4.1.3.2 All ethanol quantitation results shall be within +/- 5.0 % of the target value. All methanol, acetone, and isopropanol quantitation results shall be within +/- 10.0 % of the target value.

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

5.4.1.4 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 documented in the BAC QC file (refer to 5.4.2.8 through 5.4.2.9).

5.4.1.5 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](#).

5.4.1.6 If any of the other components do not meet all requirements in 5.4.1.3, it shall be noted in the BAC QC file (refer to 5.4.2.8 through 5.4.2.9) and no quantitative values for the failed component shall be used.

5.4.1.7 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.

5.4.2 Quality Control for Body Fluids

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

5.4.2.2 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.

5.4.2.3 Positive controls shall be selected to avoid duplicate concentrations within a sequence when possible.

5.4.2.4 Negative Control

5.4.2.4.1 Prepare a negative control sample using water according to [5.5.1.1](#) and [5.5.1.3](#) through [5.5.1.7](#).

5.4.2.4.2 Negative controls shall not contain any identifiable methanol, ethanol, isopropanol, acetone or any other identifiable volatile.

5.4.2.5 Positive Control

5.4.2.5.1 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 [5.5.1.1](#) and [5.5.1.3](#) through [5.5.1.7](#).

5.4.2.5.2 All ethanol quantitation results shall be within +/- 5.0 % of the target value. All methanol, acetone, and isopropanol quantitation results shall be within +/- 10.0 % of the target value.

- 5.4.2.6** 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 BAC QC file (refer to 5.4.2.8 through 5.4.2.9)
- 5.4.2.7** If a quality control sample in a sequence is found to be unacceptable for a component, all specimens 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.
- 5.4.2.7.1** Record the unacceptable quality control sample in the BAC QC file (refer to 5.4.2.8 through 5.4.2.9)
- 5.4.2.7.2** Notify the Blood Alcohol Key Operator or designee.
- 5.4.2.7.3** Correct any apparent problem and document the action in the instrument log.
- 5.4.2.7.4** Successful daily system check (see **5.4.1**) shall be performed prior to resuming casework.
- 5.4.2.8** 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 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.
- 5.4.2.8.1** Examples: BacQc20080818GC1-XXX or BacQc20080101GC1and2-XXX.
- 5.4.2.9** The Quality Control data packet shall include the daily system check sequence table, chromatograms for the daily system check, the completed BAC worksheet(s), the sequence table(s) for the run, the chromatograms for each control sample, and a reference to the daily run Workstation (refer to the [Toxicology Quality Assurance Procedure](#)).
- 5.4.2.10** Record each sequence in the instrument log with the date and time of the first injection and operator initials.
- 5.4.2.11 Control Charting**
- 5.4.2.11.1** Complete the Toxicology Control Chart Form and submit to the Toxicology Technical Leader.

5.5 Alcohol and Acetone Concentration in Body Fluids

5.5.1 Sampling

- 5.5.1.1** Allow all solutions and samples to be analyzed to equilibrate to room temperature.
- 5.5.1.2** Ensure that all body fluids are homogenous by shaking and/or vortexing.
 - 5.5.1.2.1** 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 **5.5.4**.
 - 5.5.1.2.2** 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 **5.5.4**.
 - 5.5.1.2.3** 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.
- 5.5.1.3** 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.
- 5.5.1.4** Prepare each liquid to be analyzed in replicate and chromatograph using a BAC method.
 - 5.5.1.4.1** The replicate set shall have the case samples pipetted and analyzed in reverse order from the first set.
- 5.5.1.5** Volatiles that elute after the internal standard 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.
- 5.5.1.6** 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.
- 5.5.1.7** The value of the multiplier in the sequence table shall be 10.

5.5.2 Identification of Volatiles

- 5.5.2.1** 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 5.3.3.2.1 for retention time window settings.

5.5.2.2 Include the sample chromatograms, calibration data, and quality control data in the case record.

5.5.2.3 Volatiles other than those listed in **5.5.2.1** may be identified using a reference material relative retention time (RRT) comparison relative to the internal standard.

5.5.2.3.1 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.

5.5.2.3.2 The reference material shall be analyzed under the same chromatographic conditions as the sample (refer to Limitation 6.3).

5.5.2.3.3 Include the sample chromatograms, the sample RRT, the reference material RRT, calibration data, and quality control data in the case record.

5.5.3 Determination of Alcohol or Acetone Concentration

5.5.3.1 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.

5.5.3.2 If any of the four measured values for an analyte are below the lowest calibrator of 0.01 g/100mL, the analyte shall be reported as negative.

5.5.3.3 If any of the four measured ethanol values are outside of +/- 5 % of the mean, the specimen shall be re-analyzed.

5.5.3.4 If any of the four measured methanol values are outside of +/- 8 % of the mean, the specimen shall be re-analyzed.

5.5.3.5 If any of the four measured isopropanol values are outside of +/- 8 % of the mean, the specimen shall be re-analyzed.

5.5.3.6 If any of the four measured acetone values are outside of +/- 15 % of the mean, the specimen shall be re-analyzed.

5.5.3.7 If subsequent reanalysis fails to meet the criteria listed in **5.5.3.3** thru **5.5.3.6**, the result shall be reported using the lowest measured value

truncated to the hundredths place. Uncertainty of measurement does not apply in these situations.

5.5.3.8 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.

5.5.3.9 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 water distribution ratio of serum:whole blood. Do not convert the uncertainty of measurement.

5.5.4 Calculations

5.5.4.1 Refer to **5.5.5** for calculations related to determination of measurement uncertainty.

5.5.4.2 Relative retention time (RRT): (analyte retention time / internal standard retention time)

5.5.4.3 Relative retention time (RRT) percent difference calculation, round to one decimal place: $[(\text{standard relative retention time} - \text{analyte relative retention time}) / (\text{standard relative retention time})] * 100$

5.5.4.4 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.

5.5.5 Uncertainty of Measurement – The uncertainty of measurement shall be calculated by the following formulas:

5.5.5.1 Ethanol confidence interval: measured concentration mean (prior to truncating) * 0.05 (ethanol process uncertainty)

5.5.5.2 Methanol confidence interval: measured concentration mean (prior to truncating) * 0.08 (methanol process uncertainty)

5.5.5.3 Isopropanol confidence interval: measured concentration mean (prior to truncating) * 0.08 (isopropanol process uncertainty)

5.5.5.4 Acetone confidence interval: measured concentration mean (prior to truncating) * 0.15 (acetone process uncertainty)

5.5.5.5 All uncertainty of measurement values will be rounded to three decimal places for reporting.

5.5.5.6 These values shall be updated annually.

5.5.6 Reporting

5.5.6.1 If only one alcohol is identified and quantitated, state the concentration in the following format required by NCGS 20-4.01(1b):

The (insert matrix) (insert analyte) concentration is (0.XX) grams of alcohol per 100 milliliters, as defined by NCGS 20-4.01 (1b). The measured (insert matrix) (insert analyte) concentration is (0.XXX +/- 0.XXX) grams of alcohol per 100 milliliters.

5.6.6.1.1 If acetone is identified and quantitated, use the statement below:

The measured (insert matrix) Acetone concentration is (0.XXX +/- 0.XXX) grams per 100 milliliters.

5.5.6.2 If more than one alcohol is identified and quantitated, state the concentration of alcohol in the format required by NCGS 20-4.01 (1b) followed by the reporting statement from **5.5.6.1** for each alcohol detected.

The total alcohol concentration is (0.XX) grams of alcohol per 100 milliliters of whole blood, as defined by NCGS 20-4.01 (1b).

5.5.6.3 If a serum conversion factor is applied to the measured alcohol concentration, add the statement below to the report.

The blood (insert analyte) concentration is (0.XX) grams of alcohol per 100 milliliters, as defined by NCGS 20-4.01 (1b). The reported blood alcohol concentration is a calculated value resulting from a converted serum alcohol concentration. The measured serum (insert analyte) concentration is (0.XXX +/- 0.XXX) grams of alcohol per 100 milliliters.

5.5.6.4 If no alcohol is identified or if any of the quantitative results of analysis before averaging are below the lowest calibrator of 0.01 grams of alcohol per 100 milliliters of whole blood, use the statement below.

The (insert matrix) alcohol concentration is 0.00 grams of alcohol per 100 milliliters, as defined by NCGS 20-4.01 (1b)

5.5.6.5 If a volatile(s) other than alcohol is/are identified use the statement below followed by the identity of the volatile(s) in addition to any required alcohol statement(s) from above.

Analysis confirmed the presence of the following substance(s): {insert the substance identified}.

- 5.5.6.6** If an analysis for other volatiles is requested and no other volatile is identified, use the statement below in addition to any required alcohol statements from above.

The analysis did not identify any other volatile substances.

- 5.5.6.7** If the blood alcohol concentration is equal to or greater than 0.080 g/100 ml before truncating and an analysis for drugs was requested, but the request did not meet the criteria set forth in **6.2**, the statement below shall be added to the report.

The blood alcohol concentration was equal to or greater than 0.08 grams of alcohol per 100 milliliters of whole blood; therefore, the requested blood drug analysis was not performed.

5.6 Alcoholic Beverage Analysis

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 solutions are homogenous by shaking and/or vortexing.

- 5.6.2** Pipette 100 μ L of the liquid to be analyzed into a 10 mL volumetric flask and dilute to volume with deionized water in duplicate.

- 5.6.2.1** Record the pipette used to make the dilution in the case record.

- 5.6.3** 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.

- 5.6.4** Chromatograph using a BAC method.

- 5.6.4.1** Volatiles that elute after the internal standard 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.

- 5.6.5** 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.

- 5.6.6** Set the multiplier in the sequence table to 10.

5.6.7 Identification of Volatiles

5.6.7.1 Refer to **5.5.2**.

5.6.8 Determination of alcohol or acetone concentration

5.6.8.1 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.

5.6.8.2 If any of the four measured values for an analyte are below the lowest calibrator of 0.01 g/100mL, the analyte shall be reported as negative.

5.6.8.3 If the sample meets the acceptance criteria for ethanol listed in **5.5.3.3**, 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.

5.6.8.4 If the sample meets the acceptance criteria for methanol listed in **5.5.3.4**, 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.

5.6.8.5 If the sample meets the acceptance criteria for isopropanol listed in **5.5.3.5**, 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.

5.6.8.6 If the sample meets the acceptance criteria for acetone listed in **5.5.3.6**, 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.

5.6.8.7 Uncertainty of Measurement – Refer to **5.5.5**.

5.6.8.8 Calculations

5.6.8.8.1 Refer to **5.5.5** for calculations related to determination of measurement uncertainty.

5.6.8.8.2 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.

5.6.8.9 Reporting

5.6.8.9.1 If alcohol or acetone is identified and quantitated, state the concentration of the analytes using the following format:

The (insert analyte) concentration is (insert concentration +/- uncertainty (both rounded to one decimal place)) percent by volume.

5.6.8.9.2 If no alcohol is identified or if the criteria in **5.6.8.2** are met, use the following statement:

No alcohol was identified.

5.6.8.9.3 If a volatile(s) other than the analytes listed in **5.6.8.1** is/are identified, add the following statement to the report:

The following other volatile(s) was/were identified: (insert the identified volatile(s)).

5.6.8.9.4 If an analysis for other volatiles is requested and no other volatile is identified, add the following statement to the report:

The analysis did not identify any other volatile substances.

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 water distribution ratio of serum:whole blood.

6.2 No further analysis shall be performed for DWI submissions with a blood alcohol concentration at or greater than 0.08 g/100 mL of whole blood, unless the case involves a death or personal injury to someone other than the driver, or the Forensic Scientist Manager approves a request from the District Attorney's office. The request must be received subsequent to the alcohol results being conveyed to the District Attorney's office, and the approval shall be documented in the case record.

6.3 The Retention Time of reference material previously analyzed using the same chromatographic conditions as the case sample can be used to identify a volatile using RRT comparison. However, if maintenance was performed that could affect retention times, the reference material shall be analyzed subsequent to the case sample.

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
03/01/2013	4	5.3.1.1: Removed-"of the last injection" 5.5.1.2: Modified to consolidate 5.5.1.3 and remove duplication with QC samples. 5.5.1.2.1 inserted Old sections 5.5.1.3, 5.5.1.4.3 and 5.5.1.4.3.1 removed due to changes in 5.5.1.2.
05/10/2013	5	4.7.1.2, 4.7.1.2.1, 4.7.2.2, 4.7.2.2.1, 4.7.3.2, 4.7.3.2.1, 4.7.4.2, 4.7.4.2.1, 4.7.5.3, 4.7.5.3.1 - simplified lot number format, change also reflected in example 5.4.3.2 - changed retention time from average of last level to average of all calibration samples 5.5.1.3.2 - removed wording due to previous changes in 5.5.1.2, Moved 5.5.1.3.2.1 to be new 5.5.1.3.2 5.5.2.9 - inserted BAC worksheet into data packet requirements 5.5.3 - removed section due to redundancy 5.6.5 and 5.7.10 - Changed "N/A" to "Currently being established."
06/14/2013	6	Definitions - added alcoholic beverage 5.4.7.6 and 5.4.7.6.1- consolidated and modified to create one range for ethanol 5.4.7.7 and 5.4.7.7.1 – consolidated and modified to create one range for other methanol, acetone, and isopropanol 5.5.1.3.2 and 5.5.2.5.2 – modified to reflect changes made in 5.4.7.6 and 5.4.7.7

		<p>Removed 5.5.2.5.2.1, 5.5.2.5.3, and 5.5.2.5.3.1 5.6.1.5, 5.7.4.1 – changed definition of late eluting volatile to “elute after the internal standard” 5.6.1.2.1, 5.6.1.2.2, 5.6.2.1 – corrected reference 5.6.3.2 – Split off from 5.6.3.1 5.6.3.3 - 5.6.3.6, and 5.7.8.3 - 5.7.8.6 – inserted acceptance criteria reflective of 99.7 % confidence interval Removed previous 5.6.3.7 , 5.6.3.7.1, 5.6.3.4, 5.6.3.5, and 5.6.4.1 New 5.6.3.7 – inserted reanalysis reporting 5.6.3.9 – removed reference to other matrices 5.6.3.10 and 6.1 – reworded ratio description Inserted 5.6.4.1 and 5.7.8.8.1 –reference to measurement uncertainty calculations Inserted 5.7.8.2 5.7.8.7 moved to 5.7.8.9 5.7.9 moved to 5.7.8.8 5.7.10 moved to 5.7.8.7 5.6.5 and new 5.7.8.7 –modified to reflect analyte measurement uncertainty determination/calculation 5.6.6 and 5.7.8.9 – Inserted Reporting statements removed from Procedure for Toxicology Analysis Inserted 6.2</p>
11/15/2013	7	Added issuing authority to header
12/18/2013	8	<p>1.0; 4.2; 4.7.3.1;4.7.4.1; and 5.3;5.4.10 – Changed wording 4.7.5.4 - Changed expiration from “three” to “six” months 5.3.1.1 - Removed and placed at 5.5.1.3.1; 5.3.1.2 removed and placed at 5.4.3.2.1 5.4.3 - Added “Using the instrument software, update the calibration table with the retention times and responses of the calibration samples.” 5.4.11 - Removed “lotadded “appropriate FA workstation” 5.5.1.3.1 which is now 5.5.1.3.2 - Removed “verification sample” 5.5.1.4; 5.5.1.5;5.5.1.6 - Changes made which condense the Daily System Check and Quality Control to one file 5.6.2.1 - Corrected the reference 5.6.2.4 and 5.6.2.5 - Removed 5.6.2.4 - Added “if a relative retention time is not available”. 5.6.6.1.1 - Added result statement for acetone 5.6.6.5 - Changed reporting statement to:(insert the identified</p>

		volatile) was/were identified 5.6.6.7 - Changed reporting statement to: "The blood alcohol concentration was equal to or greater than 0.08 grams of alcohol per 100 milliliters of whole blood; therefore, the requested blood drug analysis will not be performed."
01/24/2014	9	4.7.4.3 and 4.7.5.4 - Working Verification expiration changed to three months and Internal Standard expiration changed to six months; 5.5.2.9 - Corrected reference
06/06/2014	10	5.5.1.3.1 - moved to become 5.2.3 ; added "When maintenance is performed" 5.4.7.6, 5.5.1.3.2, 5.5.2.5.2 – changed ethanol range to +/-5 % 5.6.3 and 5.6.5 – updated acceptance criteria and uncertainty calculations to reflect updated uncertainty values
08/29/2014	11	5.3 and 5.6.3.9 – removed and added to Toxicology Quality Assurance Procedure
03/20/2015	12	4.1 – added "or equivalent" in first bullet point 4.7.1.1 and 4.7.2.1 – removed "Record the weight of each component" 4.7.5.6 and subsections – changed QC check criteria for BAC internal standard Section 5.6 is now 5.5 to correct the numbering sequence. Numbering of other sections and sections referenced within the text have been updated. 5.2.5.3.1 – changed to "replace every 12 months" 5.2.5.4.1 – changed to "Inspect every twelve months, replace as needed" 5.2.5.6 – added 5.2.5.6.1 : "Bake column prior to calibration, and as needed" and added 5.2.5.6.1.1 : "successful Daily System Check requirement after column baking." 5.2.7 – "Shutdown" replaced by "Reboot" 5.2.7.1 – removed "any"; "shutdown" replaced by "reboot" 5.2.7.2 – removed, already stated in 5.2.1 5.3.8 – removed "in the instrument log and FA"; added "on the calibration file (refer to 5.3.10)" 5.3.11 – added "the Calibration sequence table(s)" 5.3.11, 5.4.2.9 – added "refer to the Toxicology Quality Assurance Procedure" 5.4.1.7.1 – removed 5.4.1.4 – added "through 5.4.2.9" 5.4.1.6 – removed "instrument log"; added "BAC QC file (refer to 5.4.2.8 through 5.4.2.9)" 5.4.2.6 and 5.4.2.7.1 – removed "instrument log and FA"; added "BAC QC file (refer to 5.4.2.8 through 5.4.2.9)" 5.6.2.2, 5.6.2.3.3 – removed "daily system check data"; it is part of the quality control data 5.6.2.3.2 – added "(refer to Limitation 6.3)".

		<p>5.6.3.8 – removed additional space after 5.6.3.8 5.6.4.2 – removed “round to the nearest hundredth” 6.0 – added limitation 6.3 for the identification of volatiles other than ethanol, methanol, isopropanol or acetone.</p>
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