Alcohol Analysis by Headspace Gas Chromatography

1.0 Purpose - This procedure specifies the required elements and use of the headspace gas chromatograph (HS-GC) to quantitatively determine alcohol (ethanol, methanol and isopropanol) and acetone concentration in bodily fluids or other dilute solutions.

2.0 Scope – This procedure applies to Toxicology in the Raleigh (R), Triad (T) and Western (W) locations of the State Crime Laboratory.

3.0 Definitions – See Toxicology Definitions List

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Gas chromatograph equipped with flame ionization detectors with Restek BAC1 and BAC2, or equivalent 30 m x 0.53 mm capillary columns, Agilent DB-ALC-1 and Agilent DB-ALC-2, or equivalent 30m x 0.32mm column, headspace auto-sampler and data station.
- Mechanical pipette
- Volumetric flasks, Class A: 10mL (TD) size
- Liquid handler system

4.2 Materials

- Headspace vials with sealing caps
- Crimper tool

4.3 Reagents

- Deionized (DI) water

4.4 Commercial Reagents

- Helium gas – Ultra high purity grade
- Hydrogen gas – Ultra high purity grade
- Nitrogen gas – Ultra high purity grade
- Compressed air

4.5 Critical Reagents

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

4.6 Prepared Reagents

4.6.1 BAC internal standard (IS) as prepared in the **Headspace Gas Chromatography (HS-GC) Calibration and Maintenance** procedure.

5.0 Procedure

5.1 Sampling

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

5.1.2 Ensure that all solutions are homogenous by shaking and/or vortexing.

5.1.2.1 If a homogenous sample cannot be obtained due to the presence of clots or because the blood cells have been separated from the liquid, a notation shall be made in the Forensic Advantage (FA) worksheet and the reported alcohol concentration shall be calculated according to **5.4.5.3**.

5.1.2.2 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.

5.1.3 With liquid handler, deliver 1.80 mL of the IS solution and 0.20 mL of the solution to be analyzed into an appropriately labelled headspace vial and cap securely.

5.1.3.1 If analyzing an alcohol beverage, perform a dilution prior to sampling by pipetting 100 µL of the liquid to be analyzed into a 10 mL volumetric flask and diluting to volume with DI water in duplicate.

5.1.3.1.1 May be prepared by the Forensic Scientist in any amount provided that the component ratios are kept constant.

5.1.3.1.2 Record pipette and the volumetric flask used to make the dilution in the case record.

5.1.3.2 Smaller volumes may be used based upon analytical needs but shall be documented in the case record

5.1.4 Prepare each liquid to be analyzed in replicate and analyze using the most current BAC method.

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

5.2 Performance Check

5.2.1 A performance check shall be performed prior to casework in accordance with the **HS-GC Calibration and Maintenance** procedure.

5.3 Quality Control Samples

5.3.1 The first and last samples of a sequence shall be positive and negative control samples with any remaining control samples distributed throughout the run.

5.3.2 Additional positive and negative controls will be included after every 40 case samples at minimum.

5.3.3 Positive Control

5.3.3.1 Prepare the needed Commercial Multi-component Alcohol Certified Reference Material Solutions (0.050, 0.100 or 0.400 g/100mL) according to **5.1**.

5.3.3.2 Acceptable ranges for quality control samples are within a given percent of the target values. The acceptable range for Ethanol is +/-5%, all other components acceptance ranges are +/-10%.

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

5.3.4 Negative Control

5.3.4.1 A negative control shall be prepared according to **5.1** using DI water.

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

5.3.5 If a QC sample in a sequence is found to be unacceptable for a component, any specimens that are not bracketed by acceptable quality controls and contain a peak for that component in all four chromatograms that quantitate above 0.010 g/100 mL shall be reanalyzed.

5.3.6 QC Data Packet

5.3.6.1 The QC data packet shall be reviewed by a qualified Forensic Scientist and approved in the associated resource manager workstation in FA with a file name beginning with "BACQC" followed by eight digit format year/month/day and the instrument number(s). If necessary, a suffix shall be added to the name of the file to distinguish between multiple runs. (Example: BACQC20160412RGC1and2-XXX)

5.3.6.2 The QC data packet shall include the following: The completed Headspace Gas Chromatography Run Log(s), the sequence table(s) for the run and the Performance Check, chromatograms for each control sample in the run and the Performance Check and reference to the applicable workstation.

5.3.6.2.1 Record any limitations on the cover page of the QC data packet.

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

5.3.6.4 Control Charting

5.3.6.4.1 Complete the BAC Control Chart Form.

5.3.7 Storage of Instrument Files

5.3.7.1 All instrument files created during performance checks shall be placed into a compressed (.zip) file and archived in the appropriate instrument resource monthly.

5.3.7.2 All data files associated with case samples shall be placed into a compressed (.zip) file and be archived in the FA workstation object repository ("Manage Files" associated with the HSGC analysis on which it was collected.

5.4 Quantitative Determination of Alcohol and Acetone Concentration

5.4.1 Include sample chromatograms and a reference to the resource workstation that contains the associated quality control data in the case record.

5.4.2 Identification of Volatiles

5.4.2.1 Component is identified if it is integrated in the appropriate retention time window on both columns in both sample preparations.

5.4.2.2 Volatiles other than methanol, ethanol, isopropanol, and acetone may be identified according to the **Volatile Analysis by Headspace Gas Chromatography** procedure**.**

5.4.3 Determination of Alcohol or Acetone Concentration

5.4.3.1 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 BAC method. The mean of the four measured values obtained for each component is the concentration of that component.

5.4.3.2 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.

5.4.3.3 If any of the four measured values for an analyte are outside the acceptable range of the mean for a reportable component, the specimen shall be re-analyzed. Acceptable ranges are defined by the process uncertainty listed in the **Toxicology Reporting Index.**

5.4.3.4 If subsequent reanalysis fails to meet criteria as determined by the **Toxicology Reporting Index**, the result shall be reported using the lowest measured value truncated to the hundredths place. Uncertainty of measurement does not apply in these situations.

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

5.4.3.5.1 If **5.4.3.4** applies to one of the identified alcohols, the truncated lowest measured value will be used in place of the mean concentration for that alcohol.

5.4.3.6 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 according to **5.4.5.3**.

5.4.3.7 Case samples with a component that exceed the upper level of the calibration curve will be reanalyzed according to **5.1.3.2**.

5.4.4 Alcoholic Beverage Conversions

5.4.4.1 If analyzing an alcoholic beverage, the reading obtained from initial analysis will be converted to percent by volume for all components meeting acceptance criteria in **5.4**.

5.4.4.2 Reportable components will be multiplied by a conversion factor to account for the density and dilution of the beverage analyzed. The value obtained will be truncated to the tenths place. The following conversion factors shall be applied to both the concentration and the uncertainty:

- **Ethanol:** 126.7
- **Methanol:** 126.3
- **Isopropanol:** 127.4
- **Acetone:** 126.3

5.4.4.2.1 The multiplier value for alcoholic beverage analysis is obtained by multiplying the average g/100 mL result by 100 to compensate for the original dilution and dividing by the density of the appropriate analyte to convert from g/mL to mL/mL.

5.4.5 Calculations

5.4.5.1 Refer to **5.4.6** for calculations related to determination of measurement uncertainty.

5.4.5.2 Relative retention time (RRT): (Analyte retention time / IS retention time)

5.4.5.3 For 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.** Do not convert the uncertainty of measurement.

5.4.6 **Uncertainty of Measurement** – The uncertainty of measurement shall be calculated for each component (ethanol, methanol, isopropanol or acetone) individually using the following formula:

Measured concentration mean (prior to truncating) * Component process uncertainty as listed in the Toxicology Reporting Index.

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

5.4.6.2 The uncertainty of measurement values shall be updated annually.

5.4.7 **Reporting for Alcohol or Acetone Determination in Body Fluids**

5.4.7.1 All measured analyte values will be truncated to the number of decimal places specified in the appropriate reporting statement(s).

5.4.7.2 In cases where more than four acceptable values are generated for a component (ex. A case is rerun due to acetone failing to meet QC criteria, however all ethanol QCs were acceptable), the component will be reported as the average of replicate values with acceptable QCs.

5.4.7.3 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, at a coverage probability of 99.7%. (Analysis performed using HS-GC.)

5.4.7.4 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, at a coverage probability of 99.7%. (Analysis performed using HS-GC.)

5.4.7.5 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 **5.4.7.3** 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). (Analysis performed using HS-GC.)

5.4.7.6 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, at a coverage probability of 99.7%. (Analysis performed using HS-GC.)

5.4.7.7 If a clotted 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 conversion due to the clotted nature of the specimen. The measured clotted blood (insert analyte) concentration is (0.XXX +/-0.XXX) grams of alcohol per 100 milliliters, at a coverage probability of 99.7%. (Analysis performed using HS-GC)

5.4.7.8 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) (Analysis performed using HS-GC.)

5.4.7.9 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.

5.4.8 Alcoholic Beverage Reporting

5.4.8.1 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 reported to one decimal place)) percent by volume, at a coverage probability of 99.7%. (Analysis performed using HS-GC.)

5.4.8.2 If no alcohol is identified or if the criteria in **5.4.3.2** are met, use the following statement:

No alcohol was identified. (Analysis performed using HS-GC.)

5.4.9 Application of Procedure on Evidence – Insufficient Sample

5.4.9.1 If a specimen is submitted with insufficient volume for analysis, add the following statement to the report:

Quantity of specimen submitted is insufficient for analysis.

5.4.9.2 If the specimen volume is insufficient to complete the requested analysis or do any additional testing, add the following statement to the report:

Quantity of specimen submitted is insufficient for further analysis.

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 serious 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 casefile.

7.0 Safety

7.1 Refer to the State Crime Laboratory Safety Manual.

7.2 Refer to Appendix 1 for chemical hygiene and safety precautions.

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
- Performance check data
- HSGC Log Form
- Toxicology Control Chart Form
- Blood Alcohol Uncertainty Budget

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
12/01/2023	3	4.1 – removed 100 uL; removed appropriate; added system 4.4 – added grade of helium, nitrogen and hydrogen 5.1.2.1 – added Forensic Advantage 5.1.3.1.1 – added punctuation; added ratio language 5.1.3.2 - new 5.2.1 – Removed "and uploaded to the instrument manager in Forensic Advantage (FA)" 5.3.6.4.1 – Replaced "Toxicology" with "BAC" 5.3.7 – new 5.4.2.1 – updated language 5.4.3.7 – updated language and included reference 5.4.9 - new

Appendix 1

Methanol **DANGER: HIGH RISK SUBSTANCE** *		
	**HEALTH**	**2**
	FLAMMABILITY	**3**
	REACTIVITY	**0**
Detection of Release	Colorless liquid with a sweet, pungent odor.	
Signs/Symptoms of Exposure	Headache, Nausea, Dizziness, Eye damage. May cause intoxication that includes central nervous system depression, headache, dizziness, nausea, lack of coordination, and confusion.	
PEL	OSHA (TWA) 200 ppm	
Associated Hazards	Flammable. Acute oral, dermal, and inhalation toxin. Toxic if swallowed, comes in contact with skin, or inhaled. Specific target organ toxicity of eyes.	
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time less than 1minute), butyl-rubber (break through time greater than 8 hours)	
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Use explosion-proof equipment. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Dispose in Hazardous Chemical Waste. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.	

Emergency Procedures	**Eye Contact:** Flush eyes with water as a precaution. **Inhalation Exposure:** If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** After swallowing: fresh air. Make victim drink ethanol (e.g. 1 drinking glass of a 40% alcoholic beverage). Call a doctor immediately (mention methanol ingestion). Only in exceptional cases, if no medical care is available within one hour, induce vomiting (only in fully conscious persons) and make victim drink ethanol again (approx. 0.3 ml of a 40% alcoholic beverage/kg body weight/hour). **Skin Contact:** Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. **Spills:** Avoid breathing vapors, mist, or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Small spills: Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal. Large spills: Turn off sources of heat if possible; evacuate area and call 911 (Haz Mat).