Headspace Gas Chromatography (HS-GC) Calibration and Maintenance

1.0 Purpose - This procedure specifies the required elements for calibration, maintenance and use of the headspace gas chromatograph for alcohol, acetone, and volatile analysis.

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 BAC1and BAC2, or equivalent 30 m x 0.53 mm capillary columns, headspace auto-sampler and data station.
- Mechanical pipette
- Volumetric flasks
- Appropriate liquid handler

4.2 Materials

- Headspace vials with sealing caps
- Crimper tool

4.3 Reagents

- Deionized (DI) water
- Sodium chloride

4.4 Commercial Reagents

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

4.5 Primary Reference Materials – minimum ACS grade or higher

- Methanol
- Ethanol
- Isopropanol
- Acetone
- n-Propanol

4.6 Critical Reagents

- NIST traceable Multi-component Alcohol Certified Reference Material (CRM) solutions (ethanol, methanol, acetone and isopropanol) containing 0.010, 0.0200, 0.025, 0.050, 0.100, 0.200, 0.400, and 0.500 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 0.500 g/100 ml Calibration Solution

4.7.1.1 Prepare a 0.5 g/100 ml solution of methanol, ethanol, isopropanol, and acetone.

4.7.1.1.1 Weigh 0.5 g each of methanol ethanol, isopropanol, and acetone into a 100 ml volumetric flask containing approximately 25 ml deionized water.

4.7.1.1.2 Bring flask to volume with deionized water.

4.7.1.2 Labelling and storage

4.7.1.2.1 Lot Number: Eight digit format year/month/day-lab

4.7.1.2.1.1 Example: 20200408-R

4.7.1.2.2 Expiration: Three months.

4.7.1.2.3 Storage: Refrigerate

4.7.1.3 QC Check –Successful calibration that meets all acceptance criteria in 5.4.5.1 through 5.4.5.4.

4.7.2 BAC Internal Standard (IS): n-propanol

4.7.2.1 Prepare a 0.050 g/100 mL of primary reference standard n-propanol.

4.7.2.1.1 Weigh 1.0 g of n-propanol into a beaker.

4.7.2.1.2 Transfer to a 2000 mL volumetric flask.

4.7.2.1.3 Bring the flask to volume with DI water.

4.7.2.2 Add 20 g sodium chloride per 2 L of IS produced to make a 1% solution of sodium chloride.

4.7.2.3 Labelling and storage

4.7.2.3.1 Lot Number: Eight digit format year/month/day-lab

4.7.2.3.2 Example: 20160408-R

4.7.2.3.2.1 Example: 20160408-R

4.7.2.3.3 Expiration: Six months.

4.7.2.3.4 Storage: Store tightly closed at room temperature

4.7.2.4 QC Check

4.7.2.4.1 Prepare a Negative Control according to **5.3** using DI water and the new lot of IS.

4.7.2.4.2 The new lot of IS shall not contain any identifiable methanol, ethanol, isopropanol, acetone or any other identifiable volatile.

4.7.2.4.3 Place QC check data files in the appropriate Forensic Advantage (FA) resource. The data must be approved by the Toxicology Technical Leader or designee.

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:

5.1.3.1 Successful calibration (refer to **5.4**)

5.1.3.2 Successful performance checks on three separate days.

5.1.4 The data shall be filed and maintained by the Key Operator or designee 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 Following maintenance, either a successful calibration or a successful performance check shall be performed and recorded in the HSGC log before the analysis of case samples.

5.2.3 If retention time drift greater than 0.01 minutes is observed for an analyte in the performance check following maintenance, the peak retention times shall be updated to the retention times of the performance check's positive control**.**

5.2.4 Before return to service, the Key Operator or designee shall update the maintenance log when the instrument is ready to be used for casework and file any generated data in the appropriate FA instrument resource

5.2.5 Routine Maintenance

5.2.5.1 Instrument maintenance will be performed within the designated windows and on an as-needed basis. Maintenance frequency within the designated time frames should be evaluated based on the instrument conditions, past performance, and frequency of use.

5.2.5.2 Septum

5.2.5.2.1 Replace weekly when in use.

5.2.5.3 Gas Tanks

5.2.5.3.1 Ensure gas volumes are sufficient for the duration of analysis and replace as needed.

5.2.5.4 Syringe

5.2.5.4.1 Inspect prior to calibration and replace as needed not to exceed 12 months.

5.2.5.5 Liner

5.2.5.5.1 Inspect prior to calibration and replace as needed not to exceed 12 months.

5.2.5.6 Jet

5.2.5.6.1 Inspect prior to calibration and replace as needed not to exceed 36 months.

5.2.5.7 Column

5.2.5.7.1 Bake as needed.

5.2.5.7.2 Replace as needed, not to exceed 36 months.

5.2.6 Non-routine Maintenance

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

5.2.7 Reboot

5.2.7.1 Successful performance check shall be performed following GC instrument reboot.

5.3 Sampling

5.3.1 Allow all solutions to equilibrate to room temperature.

5.3.2 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.

5.4 Calibration

5.4.1 Calibration shall be performed upon preparation of a new lot of IS or after instrument maintenance that may affect the calibration (see **5.2.5.7.2 and 5.2.6**).

5.4.2 Prepare the commercial multi-component alcohol CRM solutions (0.0100, 0.025, 0.050, 0.100, 0.200, 0.400, and 0.500 g/100 mL) in triplicate according to **5.3**.

5.4.2.1 An internal 0.500 g/100 ml multi-component solution (see **4.7.1**) may be prepared and used if a commercial multi-component alcohol CRM solution is not available.

5.4.3 Using the instrument software, update the calibration table with average retention times and responses of the newly prepared calibration samples.

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

5.4.3.2 The retention time for each component shall be that of the average of all calibration samples analyzed.

5.4.3.3 The calculated peak retention time windows shall be set to the following:

5.4.3.3.1 Reference Peaks – 0.10 minutes + 1.00%

5.4.3.3.2 Other Peaks – 0.00 minutes + 1.00 %

5.4.3.4 The calibration curve shall be fit to a linear model.

5.4.4 Calibration Verification

5.4.4.1 The verification samples shall be prepared using standards from manufactures or lot numbers that differ from the ones used to prepare the calibration samples.

5.4.4.2 Prepare the commercial multi-component alcohol CRM solutions (0.020, 0.050, 0.200 and 0.400 g/100mL) in triplicate according to **5.3.**

5.4.4.3 Analyze the verification samples on the HS-GC, quantitating values with the new calibration.

5.4.5 Acceptance Criteria for Calibration and Verification Data

5.4.5.1 The calibration curves for each component shall show a coefficient of determination (r^2) of 0.995 or greater. If the calibration has a coefficient of determination of less than 0.995, appropriate action (i.e., maintenance or new solution preparation) shall be taken and the calibration repeated.

5.4.5.2 All components shall be identified by the instrument software on both columns.

5.4.5.3 The compounds shall be resolved.

5.4.5.4 Evaluate the curve by back-calculating the calibrator concentrations against the curve. Values of +/- 5% from the target concentration are acceptable for ethanol. All other analytes shall be within 10 % of the target concentration.

5.4.5.5 All verification quantitation results for ethanol shall be within +/-5% of the target concentration. Methanol, isopropanol and acetone shall be within +/-10% of the target concentration.

5.4.6 If the calibration is found to be unacceptable for any analyte, appropriate action (e.g., maintenance or new solution preparation) shall be taken and the calibration repeated.

5.4.7 Following successful calibration, the data analysis method shall be saved according to the format BACCALYear/Month/Day followed by instrument number (example: BACCAL20160408RGC1).

5.4.8 Calibration packets shall contain: The appropriate FA workstation, the calibration/verification sequence table(s), the chromatographs for each calibration and verification sample, a copy of the instrumental method, the printed calibration table, and the r^2 values of each component.

5.4.9 Calibration data shall be reviewed by a qualified Forensic Scientist and, if acceptable, approved in the appropriate FA instrument resource with a file name beginning with "BACCal" and the date in year/month/day format followed by the instrument number. (Example: BACCAL20160408RGC3)

5.4.10 The approved calibration, lot number of IS used, IS expiration date will be displayed on the front on the instrument.

5.5 Performance Check

5.5.1 A performance check shall be performed before any case sample analysis. The performance check is valid for twenty four hours after the injection of the first verification standard.

5.5.2 Prepare a 0.100 g/100 mL multi-component alcohol certified reference material solution and a negative control according to **5.3**.

5.5.3 Analyze the samples with the current BAC method.

5.5.3.1 All components shall be identified by the instrument software on both columns and be visually baseline resolved.

5.5.3.2 All performance check components shall meet the criteria in **5.4.5.4.**

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

5.5.4 Record each performance check in the HSGC log with the date and operator initials. Upload the performance check to the appropriate FA instrument resource.

5.5.5 If the performance check is found unacceptable for any analyte, appropriate action (e.g. maintenance or new solution preparation) shall be taken and the performance check repeated.

6.0 Limitations: N/A

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
- Calibration data
- HSGC Log Form
- Toxicology Reporting Index

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
06/19/2020	1	Simplifying and splitting the Headspace procedure

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).