Volatile Analysis by Headspace Gas Chromatography

1.0 Purpose - This procedure specifies the required elements for qualitative analysis of volatiles in bodily fluids using headspace gas chromatography.

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, headspace auto-sampler and data station.
- 100 µl, 1000 µl, mechanical pipette
- Volumetric flasks, Class A: 10mL, 100mL (TD) size
- Appropriate liquid handler

4.2 Materials

- Headspace vials with sealing caps
- Crimper tool

4.3 Reagents

- Deionized (DI) water

4.4 Commercial Reagents

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

4.5 Primary Reference Materials

- 1,1-difluoroethane (200 µg/mL)
- Toluene (100 µg/mL)

4.6 Prepared Reagents

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

4.6.2 **Positive Control -** Standards may be prepared by the Forensic Scientist in any amount provided that the component ratios are kept constant.

4.6.2.1 Prepare a solution containing 10 µg/ml of Toluene and 20 µg/ml of 1,1-Difluoroethane.

4.6.2.1.1 Pipette 1 ml of the toluene CRM solution and 1ml of the 1,1-difluoroethane CRM solution to a 10 mL volumetric flask and bring to volume with DI water.

4.6.2.2 **Lot number**: Eight digit format year/month/day – lab

4.6.2.2.1 **Example**: 20200319 - X

4.6.2.3 **Expiration**: n/a, prepare fresh daily

4.6.2.4 **Storage**: n/a

4.6.2.5 **QC Check**: Successful identification of components in a performance check.

4.6.3 If volatiles other than those contained are being identified, a 10 µg/ml solution will be prepared using a CRM of the suspected volatile and analyzed as an additional positive control in a batch.

5.0 Procedure

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

5.2 Performance Check and Quality Control Sample Preparation

5.2.1 Performance Check Preparation

5.2.1.1 A performance check shall be performed prior to casework to ensure that the instrument is operating properly.

5.2.1.2 The performance check will consist of running a positive and negative control.

5.2.2 Positive Control Preparation

5.2.2.1 Sample the solution prepared in **4.6.2** according to **5.3.**

5.2.3 Negative Control Preparation

5.2.3.1 A negative control shall be prepared according to **5.3** using DI water.

5.2.4 Case specimens shall be bracketed by a positive and negative control.

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

5.3 Sample Preparation

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

5.3.1.1 If a homogenous sample cannot be obtained, a notation shall be made in the FA worksheet detailing the condition of the sample and its handling.

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.

5.3.3 Prepare each case to be analyzed in replicate and analyze using the VOLRAMP method.

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

5.4 Quality Control Acceptance Criteria

5.4.1 The positive control shall identify all target compounds within the expected retention time window.

5.4.1.1 The calculated peak retention time windows shall be set to be 1% of the target compounds retention time

5.4.2 The negative control shall not contain any identified volatile substances.

5.4.3 If a QC sample in a sequence is found unacceptable for a component, any specimens that are not bracketed by acceptable quality controls and contain a reportable peak for that component in all four chromatograms shall be re-analyzed.

5.4.4 QC Data Packet

5.4.4.1 The QC data packet for the VOLRAMP method shall be reviewed by a qualified Forensic Scientist and approved in the associated resource manager workstation in FA with a file name beginning with “VOL”, 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 VOL20160531RGC3and4-XXX)

5.4.4.2 The QC data packet shall include the following: The completed Headspace Gas Chromatography Run Logs(s), the sequence table(s) for the run and the performance check, the chromatograms for each control sample and each performance check, a copy of the volatiles method and reference to the applicable workstation.

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

5.4.4.3 Record each sequence in the instrument log with the date and operator initials.

5.5 Identification of Volatiles

5.5.1 Include the sample chromatograms and a reference to the resource workstation that contains the associated QC data in the case record.

5.5.2 Identified components shall be integrated in the appropriate retention time window on both columns in both sample preparations.

5.5.3 Volatiles may be identified using a reference material RRT comparison relative to the internal standard.

5.5.3.1 The gas chromatographic RRT of the sample and reference material shall not differ by more than **1.0 %** on each column in both sample preparations.

5.5.3.2 The reference material shall be analyzed in the same analytical batch as the case specimen.

5.5.4 Calculations

5.5.4.1 Relative retention time (RRT): (analyte retention time / IS retention time)

5.5.4.2 RRT percent difference calculation, round to one decimal place: [(standard RRT – analyte RRT) / (standard RRT)] * 100

5.5.5 Uncertainty of Measurement – N/A

5.5.6 Reporting

5.5.6.1 Statements regarding qualitative volatile analysis shall be used in addition to the ones described in the **Alcohol Analysis by Headspace Gas Chromatography** procedure.

5.5.6.2 If a volatile(s) is not identified, use the statement below:

Analysis did not confirm the presence of certain volatiles, such as 1, 1 – difluoroethane and toluene. (Analysis performed using HS-GC.)

5.5.6.3 If a volatile(s) is/are identified use the statement below followed by the identity of the volatile(s)

Analysis confirmed the presence of the following substance(s): {insert the substance identified}. (Analysis performed using HS-GC.)

5.5.6.4 If a volatile(s) is requested but not identified, use the statement below.

Analysis did not confirm the presence of the following: {insert the requested substance}. (Analysis performed using HS-GC.)

6.0 Limitations

6.1 Volatiles that generally cannot be identified by current State Crime Laboratory procedures are listed in the Toxicology Reporting Index.

6.2 The VOLRAMP method is a qualitative method only. No quantitation shall be made using this method.

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).
Toxicology Unit Protocol Manual, Oklahoma SBI CSD, 2018.

9.0 Records

- Case record
- Quality control data
- Performance check data
- HSGC Log Form

10.0 Attachments – N/A

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

Appendix 1

<table>
<tr><th colspan="2">Toluene
DANGER: HIGH RISK SUBSTANCE *</th></tr>
<tr><td>  </td><td>HEALTH 2
FLAMMABILITY 3
REACTIVITY 0</td></tr>
<tr><td>Detection of Release (9.1)</td><td>Benzene-like odor</td></tr>
<tr><td>Signs/Symptoms of Exposure (2)(4)(8)</td><td>Skin irritation. May cause headache, dizziness, ataxia, drowsiness, euphoria, hallucinations, tremors, seizures, and coma if inhaled.</td></tr>
<tr><td>PEL (8)(Z Tables)</td><td>OSHA TWA 100 ppm (see Table Z-1); ACGIH Threshold Limit Value (TLV) 20 ppm</td></tr>
<tr><td>Associated Hazards (2)</td><td>Highly flammable liquid and vapor. May be fatal if swallowed and enters airways. Causes skin irritation. May cause drowsiness or dizziness. Suspected of damaging fertility or the unborn child. May cause damage to organs (Central nervous system) through prolonged or repeated exposure. Toxic to aquatic life. Harmful to aquatic life with long lasting effects</td></tr>
<tr><td>Controls (8.2)</td><td>Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Wear lab coat. Handle with gloves- nitrile break though < 1 minute – CHANGE OFTEN; fluorinated rubber break through time 480 minutes.</td></tr>
<tr><td>Safe handling, storage, disposal (7)(13)</td><td>Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep container tightly closed in a dry and well-ventilated place. Use explosion-proof equipment. Keep away from sources of ignition. Take measures to prevent the build up of electrostatic charge. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Dispose in Hazardous Waste.</td></tr>
<tr><td>Emergency Procedures (2.2)(4.1)(6)</td><td><u>Eye Contact</u>: Flush eyes with water as a precaution
<u>Inhalation Exposure</u>: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
<u>Ingestion</u>: Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.
<u>Skin Contact</u>: Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.
<u>Spills</u>: Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, soak up with inert absorbent material, and place in container. Dispose in Hazardous Waste. Large spills: Evacuate area and call 911 (Haz Mat).</td></tr>
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