IDENTIFICATION AND CONFIRMATION OF INHALANTS IN AQUEOUS AND BIOLOGICAL SPECIMENS BY HEADSPACE GAS CHROMATOGRAPHY AND HEADSPACE GAS CHROMATOGRAPHY- MASS SPECTROMETRY

47.1 METHOD

This test method may be used to identify and confirm the presence of volatile inhalant compounds in aqueous and biological specimens. Specimens are diluted with n-propanol internal standard for analysis using a headspace gas chromatograph equipped with a flame ionization detector (FID) for initial identification and a gas chromatograph equipped with a mass spectrometer (MS) detector for subsequent confirmation.

47.2 SPECIMENS

The specimen volume is 0.2 mL. Specimens include whole blood, serum, plasma, urine and vitreous humor (see 47.6.7 for analysis of tissue samples). Dilutions of specimens may be analyzed at the Forensic Scientist's discretion.

47.3 REAGENTS, MATERIALS AND EQUIPMENT

47.3.1 REAGENTS

- DI H_2O, laboratory general-use
- Inhalant compounds

  Note: Sources of inhalant compounds may vary due to the suspected inhalant and are not required to be of laboratory grade. For example, butane may be obtained from a butane lighter, difluoroethane may be obtained from a can of cleaning duster (e.g., Dust-Off®) and ethyl chloride may be obtained from a can of solvent cleaning spray (e.g., Maximum Impact® Head Cleaner).
- Methanol (MeOH), reagent grade
- n-propanol internal standard

47.3.2 MATERIALS

- GC column (Agilent HP-5MS; 30 m x 0.250 mm i.d. x 0.250 µm film thickness, or equivalent)
- GC (for headspace) J&W DBALC1 capillary column (30 m x 0.53 mm ID x 3 µm film thickness) or a J&W DBALC2 capillary column (30 m x 0.53 mm ID x 2 µm film thickness), or equivalent

47.3.3 EQUIPMENT

- Agilent 6890 gas chromatograph equipped with a flame ionization detector, or equivalent
- Agilent 7694 headspace autosampler, or equivalent
- Agilent MS (5973 or equivalent) with electron ionization source
- Airtight glass syringe for manual injection

- Cap crimper
- Hamilton Microlab 600® Autopipette, Hamilton Automatic Diluter, or equivalent
- Headspace autosampler vials (10 mL) and crimp caps
- Heating block

47.4 STANDARDS

47.4.1 Inhalant compounds are obtained as described in 47.3.1.

47.4.2 Internal standard (n-propanol) is prepared and verified according to the *Procedure for the Verification of n-Propanol Internal Standard (PTis12501)*.

47.5 CALIBRATORS AND CONTROLS

47.5.1 CALIBRATORS

47.5.1.1 As quantitation of inhalants is not performed, no calibrators are required.

47.5.2 CONTROLS

47.5.2.1 One negative control is included in the batch, containing n-propanol internal standard and DI H_2O.

47.5.2.2 At least two positive controls are included in the batch, used to bracket case specimens. Examples are listed below.

a. Where all specimens analyzed are expected to contain a single inhalant (e.g., difluoroethane), two positive controls for that inhalant are prepared (as described in 47.6.2 and 47.6.3) and analyzed, bracketing specimens in the batch.

b. Where batch specimens are expected to contain two different inhalants (e.g., difluoroethane, ethyl chloride), one positive control for each is prepared (as described in 47.6.2 and 47.6.3). One positive control is analyzed prior to specimens, and one is analyzed at the end of the batch in order to bracket specimens.

c. Where specimens are expected to contain more than two different inhalants, one positive control for each is prepared (as described in 47.6.2 and 47.6.3). One positive control is analyzed prior to specimens, and one is analyzed at the end of the batch in order to bracket specimens. The third positive control may be analyzed at any point within the batch.

d. Controls (including both positive and negative) must make up 10% of the batch (based on number of case specimens). Where more than 20 case specimens are analyzed in a batch, a third positive control must be prepared and analyzed mid-batch.

47.6 SAMPLE PREPARATION

NOTE: Independent aliquots of the specimen, controls, and blanks must be taken for initial identification and subsequent confirmation of the inhalant compound.

47.6.1 Label headspace vials for each member of the test batch (e.g., blank, negative control, positive controls, case specimens). Batch may be set up according to the following suggested sequence:

1. Blank
2. Positive Control
3. Blank to eliminate carryover
(additional blanks, as needed, to eliminate carryover)
4. Negative Control
5. Specimen*
6. Blank to eliminate carryover (as needed)
7. Positive Control
8. Blank to eliminate carryover
(additional blanks, as needed, to eliminate carryover)

*Repeat step 5 and 6 for each specimen.

NOTE: The number of blank injections following the positive control may vary, depending on the inhalant compound being injected. A sufficient number of blanks should be injected until the inhalant is not detected. Note that carryover is not likely with the HSGC acquisition method, but is more likely to occur when analyzing specimens/positive controls using the GC-MS acquisition method.

NOTE: It is advisable to run a blank after the case specimen if carryover is suspected. Multiple blanks may be run as necessary to eliminate carryover. Blanks may be analyzed at the Forensic Scientist's discretion.

47.6.2 Prepare concentrated positive control standards from inhalant sources (e.g., aerosol can):

a. Label three headspace vials for each inhalant being analyzed in the batch; one for the concentrated control (prepared directly from source) and one each for dilution 1 and dilution 2. Cap and seal the empty dilution 1 and dilution 2 vials.

   Note: Serial dilutions are utilized to prevent saturation of the volatile (specimens, inhalant, etc.) on the column and to prevent contamination.

b. Add a small amount of the inhalant into the vial labeled as the concentrated control and immediately cap and seal. Allow equilibration for at least 10 seconds before preparing dilution 1.

c. Using an airtight syringe, remove a full syringe of air (approximately 1 mL) from the equilibrated concentrated control vial and dispense into the dilution 1 vial. Allow to equilibrate for at least 10 seconds before preparing dilution 2.

d. Using an airtight syringe, remove a full syringe of air (approximately 1 mL) from the equilibrated dilution 1 vial and dispense into the dilution 2 vial.

47.6.3 Prepare the batch positive controls from the dilution 2 vial:

a. Using the auto-pipetter, aliquot 200 µL of DI H_2O and 2 mL of n-propanol internal standard into the positive control vials. Cap and seal the vials.

b. Using an airtight syringe, remove a full syringe of air (approximately 1 mL) from the dilution 2 vial and dispense into the first positive control vial. Repeat for additional positive controls, removing a fresh, full syringe of air (approximately 1 mL) for each.

47.6.4 Equilibrate case specimens to room temperature and mix before opening under a biohazard hood. Blood specimens are inspected to ensure the blood is mobile.

47.6.5 Aliquot approximately 2.2 mL DI H_2O into the vial labeled blank. Cap and seal the vial.

47.6.6 Using the auto-pipetter, aliquot 200 µL of DI H_2O and 2 mL of the internal standard solution into the negative control vial. Cap and seal the vial.

47.6.7 Using the auto-pipetter, aliquot 200 µL of the specimens and 2 mL of the internal standard solution into the respectively labeled vials. Cap and seal each vial.

Note: Non-homogenized tissue specimens (such as a lung section), may be received in sealed bags or septum jars. Prepare a vial in the same manner as a negative control, sample a full syringe of air from the septum jar, and inject into the appropriately labeled headspace vial. It may be necessary to open the bag/jar and remove a small sample of the lung, adding it to the headspace vial. Section sampling of the tissue samples will be documented in the batch paperwork.

47.6.8 Between each aliquot, rinse and wash the pipette tip appropriately (e.g., rinse pipette tip with diluted bleach and/or DI H_2O. Repeat if necessary.)

47.7 INSTRUMENTAL PARAMETERS (HSGC)

- Load and edit sequence on the headspace gas chromatograph. Enter the blanks, controls and specimens into the sequence table. All samples are identified as Sample in the Sample Type column.

- Place each headspace vial in its respective position on the headspace autosampler and verify this placement against the sequence log.
- Acquisition method – HSGC: BLDALCO for all. [Note: The method name may contain a numeric suffix to differentiate between instruments; for example BLDALCO1 for headspace instrument 1. A copy of the acquisition method for each headspace instrument is available at the instrument.]

47.8 VIAL PREPARATION/INSTRUMENTAL PARAMETERS (GC-MS)

- Heat prepared headspace vials to approximately 70˚C prior to injection into the GC-MS.
- Using an airtight glass syringe, remove approximately 1mL of the headspace air from the headspace vial and manually inject the sample into the GC-MS inlet.
- Acquisition method – GC-MS: VOL for most compounds. Compounds with a later elution time may be analyzed using the VOL-LONG method.

47.9 DATA ANALYSIS

For HSGC, printed reports for each vial in the batch are generated for review, along with a copy of the sequence table and worklist. For GC-MS, chromatograms are printed for each injection, with mass spectral matches printed for target compound peaks in controls and specimens.

47.10 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

47.10.1 Blank

The blank injection at the beginning of the batch, and any blanks directly preceding specimens, shall be devoid of any significant peaks[1,2]. These criteria do not apply to blanks run after positive controls/semi-quant calibrators for the purpose of eliminating carryover.

47.10.2 Controls/Semi-quant Calibrators

- The negative control(s) shall be devoid of any significant peaks other than n-propanol[2]. Identification is based on acceptable retention time matching and an integrated, symmetrical peak. All negative controls must meet these criteria for the batch to be accepted.
- The positive controls shall contain peaks for n-propanol and the inhalant compound in question[2].

[1] Peaks appearing in the blank, calibrators and negative controls that are fully resolved from the inhalant compound or internal standard are considered extraneous and not significant.
[2] On the GCMS, each spectrum will show a large air peak at the beginning of each run. This is not considered a significant peak.

47.11 CRITERIA FOR SPECIMEN ACCEPTANCE

If the criteria for batch acceptance have been satisfied, the results of individual case samples are deemed suitable for reporting if the following criteria are demonstrated:

- Any chromatographic peak for the inhalant compound and n-propanol shall appear symmetrical.
- The retention time for the inhalant compound and n-propanol are ±2% of those in the first positive control injected. These are inclusive ranges.
- A mass spectral match of the detected compound is compared to that in an approved mass spectral library or to a mass spectrum from the positive control. When using a library match, spectrum agreement should be 75 or greater wherever possible, taking into consideration the appearance and abundance of ions specific to that compound (an extracted ion match may be necessary). A reference spectrum for the compound found in a published article, research paper or other reference material may be acceptable if the electronic library match is not feasible, provided the source is documented.

47.12 REPORTING

All inhalant compounds are reported qualitatively. Individual test results from the HSGC and GC-MS test methods will be entered in LIMS (use HSGC-MS designation in LIMS when entering GC-MS confirmation results).

47.13 DOCUMENTATION AND REVIEW

- Analysts will batch their chromatograms, sequence tables (HSGC), and spectral matches together and submit the batch for both technical and administrative review. The reviewer will verify that the batch contains all members of the batch, and that all dates are correctly documented. The reviewer will also verify that the batch meets the criteria for batch acceptance in 47.10.
- The reviewer will sign and date the batch, indicating that the batch file is complete and the above procedures have been reviewed.
- Upon completion of the technical and administrative review, the batch is returned to the analyst.
- The final batch file shall contain the batch documentation, including positive and negative controls and all relevant sequence tables and chromatograms. Blanks run directly prior to specimens are filed with the specimen which they precede.

APPENDIX A - INSTRUMENTAL PARAMETERS

HSGC Parameters

Split/Splitless Inlet	
Mode	Split
Inlet Liner	4 mm splitless w/glass wool plug
Split Ratio	1:1
Inlet Temperature	250°C
HS Oven Temp	70°C
HS Loop Temp	85°C
HS Transfer Line Temp	125°C
Vial Equilibration	10 min

Oven/Column/Detector	
Carrier Gas Mode	Constant Flow
Carrier Gas Flow	16 mL/min
Temperature (constant)	40 °C
Run Time (BLDALCO)	2.5 min
Run Time (VOL-LONG)	10.0 min
GC Cycle Time	3 min
FID Detector Temp	250°C

GC-MS Parameters

GAS CHROMATOGRAPH

Split/Splitless Inlet	
Mode	Split
Inlet Liner	4 mm splitless w/glass wool plug
Split Ratio	30:1
Temperature	250°C

Oven/Column	
Carrier Gas Mode	Constant Flow
Carrier Gas Flow	1.5 mL/min
Gas Type	Helium
Initial Temperature	50 °C
Run Time (VOL)	3.0 min
Run Time (VOL-LONG)	10.0 min

MASS SPECTROMETER

Solvent Delay	0.25 min	MS Quad Temperature	150 °C
EM Offset	Set in tune	MS Source Temperature	230 °C
Acquisition Mode	Scan		

LIST OF CHANGES

Revision Date	Description	Page Number
11/15/21	This procedure describes the testing of inhalants in aqueous and biological specimens with updated acquisition method. The Miscellaneous Volatiles SOP (TCmv12726) will be archived with the effective date of this new procedure. See DRA dated 5/18/21.	All