Helium Analysis by Gas Chromatography with Thermal Conductivity Detection

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1 INTRODUCTION

Asphyxiation with helium, both as a means of suicide and accidentally in practitioners of autoerotic asphyxia, is sometimes encountered in death investigations. Breathing pure helium produces almost no secondary physiological responses when compared to other common asphyxiants such as nitrogen (narcosis) and carbon monoxide (severe nausea), and compressed helium is readily available from party supply stores.

2 SCOPE

Analyses	☒ Screening ☐ Confirmation ☐ Quantitation
Matrices	Biological and non-biological samples
Analytes	Helium
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Thermal conductivity detectors (TCD) function by measuring the change in temperature of a heated wire placed at the exit of a gas chromatography (GC) column that occurs when an analyte with a thermal conductivity different than that of the carrier gas exits the GC column. Normally either hydrogen or helium is used as a carrier gas with a TCD, since these gases have much higher thermal conductivity (at least 3-fold greater at 500 K) than any other gases, providing strong response. For this method, the normal practice is reversed, and nitrogen, with a moderate thermal conductivity (38 W/K at 500 K), is used as carrier gas, providing high sensitivity for helium (222 W/K at 500 K). Specificity is enhanced by the fact that relatively few gases have higher thermal conductivities than nitrogen, meaning that only a few compounds can produce interfering signals. At 500 K, the only common room-temperature gases with thermal conductivity differences greater than 5% of that for helium are: ammonia (7%), ethane (8%), ethylene (6%), hydrogen (>100%), methane (15%), and neon (17%).

4 SPECIMEN CRITERIA

This procedure can be performed on a variety of biological fluids and tissue samples. Specimens for this exam should be collected as soon as possible after death and must be kept under a gas-tight seal until they are analyzed. For fluid specimens, the preferred container is a vacuum-sealed blood collection tube approximately 2/3 to 3/4 full. A 20 mL crimp-top headspace vial is suitable for small tissue samples. For large tissue samples, the best container is a new metal paint can of the smallest size necessary to contain the sample.

5 EQUIPMENT

5.1 Equipment

A.	basin or other container suitable for filling with water and inversion of flasks
B.	centrifuge
C.	hammer and metal probe/punch
D.	heating block with thermometer
E.	vacuum source
F.	volumetric flasks (100-mL)

5.2 Consumables

A.	16 x 100 mm disposable glass culture tubes
B.	electrical tape (or another well-sealing adhesive tape)
C.	fold-over rubber septa
D.	standard GC syringe, 10 µL
E.	syringe filters (25 mm 0.22µm PTFE)
F.	syringe needles (various sizes)

5.3 Instruments

5.3.1 Agilent 6890N Gas Chromatograph

5.3.1.1 Detectors

Thermal Conductivity Detector

5.3.1.2 Column

J&W HP-Molesieve 30 m x 0.32 mm x 12 µm, or equivalent

5.4 Software

Windows XP SP2; Chemstation E.02.00.493; Xcalibur 2.0.7 SP1

5.5 Chemicals/Reagents

5.5.1 Purchased

A.	Deionized water	(Laboratory supply or purchased)

5.6 Standards/Controls

5.6.1 Purchased

A. Compressed Air Supply	Laboratory supply/purchased
B. Drug-Free Blood	Obtained from Cliniqa or an equivalent approved supplier.
C. High Purity Helium	> GC-grade

5.6.2 Prepared

A. Air Standard

An air standard serves to demonstrate the absence of helium or target analyte in the source of air used for the procedure. The air standard is made by filling a 100 mL volumetric flask completely with deionized water. The flask is then inverted in a deionized water bath and the water is displaced by air taken from the laboratory compressed air supply. The flask is then capped with a fold-over rubber septum while still inverted in the water bath. Alternatively, an air standard may be obtained by simply sampling the ambient atmosphere with a standard 10 µL GC syringe.

B. High Purity Helium Standard

The high purity helium standard is analyzed to demonstrate that the target analyte source material is free of interferences and as a source for creating the mixed helium/air control. It is prepared in the same manner as the Air Standard, substituting a high purity helium source for the laboratory compressed air supply.

C. Mixed Helium/Air Control (1% helium in air)

Prepare an air standard as in (A) above. Then use a 3 mL syringe with a fine-gauge needle to transfer 1.0 mL of the high purity helium standard (B) into this flask. A sample of this control is analyzed prior to each batch of samples to demonstrate that the instrument is performing properly.

D. Negative Control

Measure 9 mL of drug-free blood into a 16 x 100 mm culture tube and cap with a fold-over rubber septum. Centrifuge at low speed (≤1000 rpm) for 5 min and, using a fine-gauge needle, vent the tube headspace to the laboratory vacuum system for about 5 s. A negative control will be analyzed with every batch of samples.

E. Positive Control

Measure 9 mL of drug-free blood into a 16 x 100 mm culture tube and cap with a fold-over rubber septum. Run a long large-gauge needle through the septum into the blood sample and use a short fine-gauge needle, with an attached syringe filter, to vent the tube headspace. Gently bubble high-purity helium through the blood sample

for about 30 min. The blood sample will generate copious quantities of foam, and the vent needle must be equipped with a syringe filter to prevent the sample from foaming out of the tube. After this sparge, centrifuge the sample at low speed (≤1000 rpm) for 5 min and, using a fine-gauge needle, vent the tube headspace to the laboratory vacuum system for about 5 s. A positive control will be analyzed with every batch of samples.

6 PROCEDURE

	Step	Note	Reference/Lot
☐	A. Where possible, centrifuge samples at low speed (≤1000 rpm) for 5 min prior to equilibration and analysis. This will help prevent contamination of the sampling syringe with biological material.		
☐	B. Place all controls and unknowns into a laboratory heating block set at approximately 36°C to equilibrate the headspace. The container type for the unknown samples may preclude placement in a heating block in which case another suitable equilibration method should be substituted. Equilibrate for at least 30 minutes.	2	
☐	C. While the samples are equilibrating, perform the necessary QC checks for the GC-TCD instrument. The Air Standard, the High Purity Helium Standard, and the Mixed Helium/Air Control will be analyzed at this time to verify proper instrument performance.	3	
☐	D. Using a 10 µL standard GC syringe, sample the headspace of each control or unknown and analyze using the conditions given below. For headspace vials and Vacutainer (or similar) tubes, the headspace is sampled directly through the container septum. For specimens in paint cans (or similar containers), use a hammer and a metal punch or probe to make a pinhole in the lid of the container. Immediately cover this hole with a double layer of electrical tape, and then sample the headspace through the electrical tape.		

7 ANALYTICAL PARAMETERS

7.1 Agilent Gas Chromatograph

7.1.1 Oven – Standard Conditions

Step	Temperature (°C)	Hold (min)	Ramp (°C/min)
1	35	10	0 (isothermal)
Equilibrium Time (min)	0.2		

Total Run Time (min): 10.00

7.1.2 Inlet/Carrier/Column

Inlet		Carrier		Column	
Temperature (°C)	200	**Gas**	Nitrogen	**Type**	HP-Molesieve
Injection Mode	Split (Manual)	**Mode**	constant flow	**Length (m)**	30
Split Ratio	2:1	**Flow (mL/min)**	1.0	**Internal Diameter (mm)**	0.32
				Film Thickness (µm)	12

7.2 Thermal Conductivity Detector

Temperature (°C)	250
Polarity	negative
Data Sampling Rate (Hz)	5
Reference Gas	nitrogen
Reference Flow (mL/min)	20
Make Up Gas	nitrogen
Make Up Flow (mL/min)	5.0

8 DATA ANALYSIS

8.1 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Evaluation of results should be based upon comparison of analytical data for an unknown sample to data from analysis of positive and negative control samples.

8.1.1 GC-TCD Performance Criteria

The Air Standard should be free of helium or target analyte. The Helium Standard (or target standard) should be free from other interferences. The air peak and the helium (or target) peak should be well-resolved.

8.1.2 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs.

Additionally, the following two criteria should be met.

8.1.2.1 Retention Time

The retention time of the presumptive helium peak should be within ±2% of the retention time obtained from injection of a positive control sample.

8.1.2.2 Signal-to-Noise

To justify the existence of a peak, its signal-to-noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10-fold-greater than that for any observed peak at similar retention time in a negative control injected just prior to the sample.

9 REPORTING

9.1 Detection

Results of this analysis are exclusionary in nature. At present, there is no method available to confirm presumptive positive helium results. Appropriate caution should be used in reporting presumptive positive analytical results.

10 CORRECTIVE MEASURES

10.1 HS-GC-TCD

The GC column used in this procedure is a molecular sieve column, which may retain water. The column may be reconditioned by heating the GC oven to 225°C for >4 hours or overnight. Insufficient column conditioning results in poor chromatographic separation between the target peaks.

11 PERFORMANCE CHARACTERISTICS

11.1 Limit of Detection

This method will detect helium at a level of 0.5% v/v in air standards. The response for a 1% v/v standard of helium in air is less than 5% of that observed for the positive control blood specimen.

11.2 Carryover

No carryover was detected during validation.

12 LIMITATIONS

12.1 Matrix Interference

None known

13 SAFETY

Take standard precautions for the handling of chemicals and biological materials. Refer to the FBI Laboratory Safety Manual for guidance. When preparing the positive control blood sample, ensure that a syringe filter is attached to the vent needle in order to prevent aerosol formation from the blood sample.

14 REVISION HISTORY

Revision	Issued	Changes
03	07/15/2022	Complete document reformat