Volatile Chemicals by Headspace GC/MS(EI)

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1 INTRODUCTION

The analysis of volatile chemicals is performed by headspace gas chromatography. This technique is based on various gas laws which state that when a volatile liquid in solution comes into contact with a closed air space, an equilibrium forms between the liquid phase and the headspace. At a given temperature, the partial pressure of the volatile in the headspace is directly proportional to its concentration in solution. This method affords a means of analyte separation from the biological matrix and produces a ready-made vapor for chromatographic analysis.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☐ Quantitation
Matrices	Blood, serum, plasma, urine, vitreous humor, tissue homogenate, foods, beverages, solid/liquid unknowns
Analytes	See Section 11.1 for a list of validated analytes
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

An aliquot of sample or control is combined with internal standard and sodium chloride in a headspace vial. The vial is heated for 30 minutes and then the headspace is analyzed by gas chromatography with mass spectral detection (GC/MS).

4 SPECIMEN CRITERIA

This procedure can be performed on a biological fluid such as: blood, serum, plasma, urine, vitreous humor, or tissue homogenate. When available, a minimum of 0.25 mL of specimen is used in this assay. This procedure may also be performed on foods, beverages, or unknown solid or liquid samples, although dilution may be required for samples with high amounts of volatile chemicals present.

5 EQUIPMENT

5.1 Equipment

A. Headspace vial cap crimper
B. Vortex mixer
C. Volumetric flasks (100-mL and 1000-mL)
D. Pipette (Adjustable or 0.5 mL fixed)
E. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.

5.2 Consumables

A. 20-mL or 10-mL disposable headspace vials
B. Headspace vial magnetic caps

5.3 Instruments

Agilent Gas Chromatograph/Mass Spectrometer

5.3.1 Column

30 m x 0.25 mm x 1.4 µm DB-624, or equivalent

5.3.2 Autosampler

Gerstel CombiPal

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1
GC/MS	Enhanced Chemstation	E.02.02.1431
Autosampler	Gerstel Maestro 1	1.5.3.3/3.5
Data Analysis	Xcalibur	2.0.7 SP1

5.5 Chemicals/Reagents

5.5.1 Purchased

A. Sodium Chloride	ACS Reagent Grade
B. Deionized Water	Laboratory Generated/Purchased

5.5.2 Prepared

Saturated sodium chloride solution (brine solution):
To a 500 mL volumetric flask, add 450 mL deionized water and 175 g sodium chloride. Gently heat with continuous stirring for at least one hour. Remove the stirbar, fill to volume with deionized water, and mix by inversion. A small amount of undissolved solid should remain in the bottom of the flask. Store in glass at room temperature. Stable for one year.

5.6 Standards/Controls

5.6.1 Purchased

A. Acetone	HPLC Grade
B. Acetonitrile	HPLC Grade
C. Ethanol	200 proof, pharmaceutical grade
D. Isopropanol	HPLC Grade
E. Methanol	Reagent Grade

5.6.2 ***Prepared***

A.	Acetonitrile Internal Standard Solution (0.08% (w/v))
	Add 100 µL acetonitrile to about 90 mL deionized water in a 100 mL volumetric flask. Dilute to the mark with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass container. Stable for 6 months.
B.	Volatile Injection Solution / Positive Control (0.01 % v/v of each component)
	Prepare by adding 500 mL of deionized water into a 1000 mL volumetric flask. Add 0.1 mL each of methanol, ethanol, isopropanol, and acetone. Bring to the mark with deionized water. Store refrigerated in a tightly-sealed glass or plastic container. Stable for at least two years. This Injection Solution may be analyzed as the Positive Control for the assay. Another suitable positive control may be analyzed, as appropriate.
C.	Whole Blood Volatiles Control
	Purchased from Cliniqa. Contains acetone, ethanol, isopropanol and methanol.. Storage conditions and stability determined by manufacturer. This Volatiles Control may be analyzed as the Positive Control for the assay. Another suitable positive control may be analyzed, as appropriate.
D.	Negative Control
	An appropriate negative matrix should be used as the Negative Control. In the absence of a more appropriate matrix, deionized water may serve as the negative control for this analysis.

6 PROCEDURE

	Step	Note	Reference/Lot
	A. Samples/Controls		
	1. To labeled 20 mL headspace vials add:		
☐	i. 0.5 g sodium chloride or 2.0 mL saturated sodium chloride solution		
☐	ii. 0.5 mL (g) of specimen(s) / control(s)		
☐	iii. Negative Control(s)		
☐	iv. Positive Control(s)		
	Alternatively, ½ of the stated amounts may be used if 10 mL headspace vials are used.		
	Notes: Unknown liquids may have to be diluted before analysis. All samples in a batch should be prepared the same way.		
	B. Internal Standard(s)		
☐	1. Add 500 µL of Acetonitrile Internal Standard Solution		
	☐ i. The acetonitrile internal standard solution may be omitted or substituted if acetonitrile is a suspected target analyte.		
☐	2. Immediately cap and crimp with headspace vial.		
☐	3. Vortex for 10 seconds.		
☐	**C. Analyze by HS-GC-MS**		

7 ANALYTICAL PARAMETERS

7.1 Agilent Gas Chromatograph

7.1.1 Oven – Standard Conditions

Step	Temperature (°C)	Hold (min)	Ramp (°C/min)
1	50	3	10
2	250	21.5	

Total Run Time (min): 44.5

7.1.2 Inlet/Carrier/Column

Inlet		**Carrier**		**Column**	
Temperature (°C)	150	**Gas**	ultrapure helium	**Type**	DB-624
Injection Mode	Split	**Mode**	constant pressure	**Length (m)**	30
Split Ratio	10:1	**Pressure (psi)**	6.54	**Internal Diameter (mm)**	0.25
				Film Thickness (µm)	1.40

7.2 Agilent Mass Spectrometer

	Standard Conditions
Ionization Mode	Electron Impact (+)
Scan Mode	Full Scan
Scan Range (m/z)	27-400
Multiplier Offset (v)	106
Solvent Delay (min)	1.6
Temperatures (°C)	
Source	230
Quadrupole	150
Transfer Line	260

7.3 Autosampler

Mode	Headspace	Syringe	
Incubation		Temperature (°C)	90
Temperature (°C)	80	Sample Fill Volume (mL)	1.0
Time (min)	30	Sample Fill Rate (mL/sec)	0.5
Agitator Speed (rpm)	300	Sample Fill Strokes	5
Agitator Timing (s) On/Off	10/1	Sample Injection Speed (mL/sec)	1.0
Cycle Time (min)	48	Flush Time (min)	4.0

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. In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard, calibrator, or Positive Control.

8.1.1 Batch Acceptance Criteria

No analytes of interest should be detected in the Negative Control. For this purpose, analytes of interest are defined as those analytes that will be reported for this batch.

Each of the analytes in the Positive Control should be detected in the headspace GC/MS data. If a targeted run is being performed for a limited set of analytes, only those analytes need to be detected in the Positive Control.

8.1.2 Unknown Sample Acceptance Criteria

8.1.2.1 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.

8.1.2.1.1 Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or Positive Control. The relative retention times of the components should agree with those listed in the enclosed table within ±2%. If not, the shift in relative retention times should be noted and appropriate corrections made when analyzing the data generated from case specimens.

8.1.2.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak 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 or blank injected just prior to the sample.

8.1.2.2 Mass Spectrometry

The mass spectrum of the analyte of interest should compare to a reference standard, extracted calibrator, or an extracted Positive Control. See the Guidelines for Comparison of Mass Spectra (TOX-104) for further guidance.

Table 1: Approximate Relative Retention Times (RRT) to Acetonitrile on DB - 624 column

Chemical	RRT	Chemical	RRT	Chemical	RRT
Acetaldehyde	0.606	Diethylamine	1.203	1,4-Dioxane	2.241
Methanol	0.629	Hexane	1.250	Isoamyl Alcohol*	2.584
Pentane	0.783	1-Propanol	1.321	Toluene	2.635
Ethanol	0.800	Ethyl Acetate	1.530	Ethyl Benzene	3.305
Diethyl Ether	0.838	Chloroform	1.625	m/p-Xylene	3.359
Acetone	0.919	n-Butyl Chloride	1.739	o-Xylene	3.542
Isopropanol	0.951	Isobutyl Alcohol*	1.794	2,2,2-Trichloroethanol	3.894
Acetonitrile	1.000	Benzene	1.846	Octanol	4.688
Methylene Chloride	1.057	Isooctane	1.875	Cresol	5.067
t-Butanol	1.082	Butyl Alcohol*	2.062		

*These alcohols were analyzed as nitrite standards.

8.2 Calculations

8.2.1 Software

Qualitative calculations may be performed by one or more of the following software packages:

A. Thermo Xcalibur
    1. QualBrowser
    2. QuanBrowser
B. Agilent Masshunter
C. Microsoft
    1. Excel

9 Reporting

Care should be taken when interpreting results from grossly decomposed or putrefied samples, as well as samples that have been embalmed. Severe or extensive putrefaction will result in the generation of a wide variety of low molecular weight volatile compounds in biological specimens, including ethanol, higher weight n-alcohols, aldehydes, sulfides, mercaptans, and alkylamines.

9.1 Measurement Uncertainty

Refer to CHEM-100 and TOX-101.

10 Corrective Measures

Refer to TOX-101 for guidance on action steps in the event of a quality control failure.

11 PERFORMANCE CHARACTERISTICS

11.1 LOD

Analyte	LOD (%v/v)
1,4-dioxane	0.0100
2,2,2-TCE	0.0050
Acetaldehyde	0.0100
Acetone	0.0050
Benzene	0.0001
Butyl alcohol	0.0050
Chloroform	0.0010
Cresol	0.0100
Diethyl ether	0.0010
diethylamine	0.100
Ethanol	0.0100
Ethyl acetate	0.0010
Ethyl benzene	0.0010
Hexane	0.0100
Isoamyl alcohol	0.0010
Isobutyl alcohol	0.0050
Isooctane	0.0100
Isopropanol	0.0050
Methanol	0.0050
Methylene chloride	0.0001
n-butyl chloride	0.0010
Octanol	0.0010
Pentane	0.0100
Propanol	0.0100
t-butanol	0.0010
Toluene	0.0002
Xylenes	0.0010

11.2 Carryover

No significant carryover identified during validation.

12 Limitations

12.1 Interferences

None known.

12.2 Processed Sample Stability

The following compounds are not stable when processed samples sit for one week at room temperature: methylene chloride, hexane, n-butyl chloride, toluene, octanol, xylenes, and ethyl benzene. Therefore, it is suggested that samples be processed the day of analysis, when possible.

13 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 Revision History

Revision	Issued	Changes
04	02/11/2022	Document reformat. Minor changes to introduction, scope and other language.