## Name of Procedure:

Toxicology
Determination of Alcohol and Volatiles in Body Fluids and Other Dilute Solutions by Headspace Gas Chromatography.

## Suggested Use:

To determine the alcohol concentration in human body fluids or other dilute solutions. May also be used to qualitatively determine other volatile compounds present in body fluid or dilute solutions.

## Introduction:

Quantitative analysis by headspace gas chromatography is possible because of the relationship given by Henry's law, P/M = constant, which states that for dilute solutions the vapor pressure (P) of a volatile solute over the solution at a constant temperature is directly proportional to the mole fraction (M) of the solute. By utilizing a series of calibration standards the concentrations of ethanol, methanol, acetone, and isopropanol in body fluids and dilute solutions can be determined by headspace gas chromatography. Other volatiles may be identified by first calibrating or utilizing the appropriate standards on both columns.

## Apparatus and Chemicals Needed to Perform Procedure:

1: LEAP Technologies HS500 Static Headspace Autosampler.
2: Varian 3800 gas chromatograph.
3: Restek BAC1 (Front) and BAC2 (Middle), 30m, 0.53 mm (i.d.) capillary columns.
4: Personal computer equipped with the Varian Star Chromatography Workstation software.
5: Volumetric glassware:
Volumetric flasks - 100, 1000, and 2000 ml (TC) sizes.
Volumetric pipets - 8, 10, 20, and 50 ml (TD) sizes.
6: Hamilton-Microlab 1000 plus, Hamilton 530B diluter/dispenser, or other appropriate liquid handler.
7: 10 mL Headspace vials with sealing caps.
8: Crimper tool.
9: Ethanol
10: n-Propanol

11: Isopropanol (IPA)
12: Acetone
13: Methanol
14: Helium gas
15: Hydrogen gas
16: Compressed Air
17: gas tight syringe

**Formula for Preparing Reagents and Expiration Dates of Reagents:**

1: Stock Solutions:

(A) Stock Calibrator Solution
Prepare a 1.000 gram/100 ml solution of ethanol, methanol, acetone, and isopropanol. Place the date on the container. Stock calibrator solution should be refrigerated and prepared annually.

Example: Weigh out 10 g of ethanol, methanol, acetone, and isopropanol into a beaker. Decant each into a single 1000 ml volumetric flask. Dilute the flask to volume with deionized water.

(B) Stock Verification Solution - Prepare a 1.000 gram/100 ml solution of ethanol, methanol, acetone, and isopropanol. Place the date on the container. Stock verification solution should be refrigerated and prepared annually.

Example: Weigh out 5 g of ethanol, methanol, acetone, and isopropanol into a beaker. Decant each into a single 500 mL volumetric flask. Dilute the flask to volume with deionized water.

2: Working Solutions:

(A) Calibrator Solutions - Prepare 0.080, 0.200, and 0.500 gram/100 ml calibrator solutions. Place the dates on the containers. Calibrator solutions should be replaced every 3 months.

Example: Pipet 8, 20, and 50 ml respectively of the stock calibrator solution into 3 separate 100 ml volumetric flasks. Dilute each flask to volume with deionized water.

(B) Internal Standard - Prepare a 0.050 gram/100 ml n-propanol internal standard solution. Place the date on the container. Internal standard solution should be replaced every 3 months.

Example: weigh out 1 gram of n-propanol into a beaker. Decant into a 2000 ml volumetric flask. Dilute the flask to volume with deionized water.

(C) Verification Standard - Prepare a 0.100 gram/100 ml verification solution. Place the date on the container. Verification solution should be replaced every 3 months.

Example: pipet 10 ml of stock verification solution into a 100-ml volumetric flask. Dilute the flask to volume.

(D) Commercial Verification Standard - An ethanol standard acquired from an outside commercial source with a concentration between 0.050 g/100 mL and 0.500 g/100 mL, inclusive.

## Application of Procedure on Evidence and Quality Control Check:

1: Varian 3800 gas chromatograph set up:

The method parameters can be stored in memory of the Varian 3800 (see Varian 3800 Operating Manual for detailed assistance) or can be activated from methods stored in the computer. Allow the Varian 3800 to equilibrate before use.

Method Parameters for alcohol determinations:

Initial Column Temp (40$^{o}$C) Isothermal
Injector Temp (200$^{o}$C)
Detector Temp (200$^{o}$C)
Column Flow 12.5 ml/min. (Nominal)
Split 1:2

Method parameters for determination of other volatiles

A temperature programmed method may be used to determine qualitatively other volatiles by utilizing the appropriate standards on both columns.

2: HS500 Static Headspace Autosampler set up:

The following parameters are stored in memory of the HS500 Autosampler (see LEAP's HS500 Static Headspace Autosampler Operating Manual for detailed assistance). The left and right arrow keys can be used to move from one parameter to another. To adjust a value in a selected parameter use the up and down keys.

Method Parameters:

INJECTION MODE - NORMAL INJECTION
INCUBATION TEMP. 070°
INCUBATION TIME HH:MM:SS 00:06:00
AGITATR CYCLE RUN 06 STOP 06
AGITATR SPEED 1600 RPM
SAMPLE EXTRACTION CNT 1
DEFAULT RUNTIME HH:MM:SS 00:04:00
SYRINGE HEATER TEMPR 070°
VOLUME SAMPLE x1.0ul 1000
SYRINGE FILLING CNT 00 DELAY 00:01
PLSPD x1ul/sec FILL 0333 INJ 1000
INJECTION POINT OUTER
SPLIT TIME PRE 00:00 PST 00:00
INJ DELAY xO.1sec PRE 00 PST 00
SYRINGE BKOUT INCR 05° MM:SS 00:10
SYRINGE FLUSHING MM:SS 01:30

3: Varian Star Chromatography Workstation software set up:

The parameters are stored under the method utilized to perform the analysis. Example bacleft.mth and bacright.mth.

Module Address: 44 (left) *or 45 (right)*

Valve Table

Front Injector Type 1079
Oven Power: On
Coolant: Off

Front Injector EFC Type 1

| Time (Min) | Split State | Split Ratio |
|---|---|---|
| Initial | On | 2 |

Column Oven

Coolant: Off
Stabilization Time: 0.00 min

| Temp (C) | Rate (C/min) | Hold (min) | Total (Min) |
|---|---|---|---|
| 40 | 0.0 | 4.00 | 4.00 |

Front *and Middle* FID Detector

Oven Power: On
Temperature: 200 C
Electronics: On
Time Constant: Fast

| Time | Range | Autozero |
|---|---|---|
| Initial | 11 | Yes |

Front *and Middle* Type 11 Detector EFC

Make up flow: 18 ml/min
H2 flow: 30 ml/min
Air Flow: 300 ml/min

Output Port A *(B and C)*

| Time (min) | Signal Source | Attenuation |
|---|---|---|
| Initial | Front | 1 |

Data Acquisition

Detector Bunch Rate : 4 points (10.0 Hz)
Monitor Length: 64 bunched points (6.4 seconds)
Front FID/TSD Scale: 1 Volts
Middle FID/TSD Scale: 1 Volts
Rear FID/TSD Scale: 1 Volts

Integration Parameters Address 44 *or 45* Channel Front *or Middle*
Subtract Blank Baseline : No
Initial S/N Ratio : 5
Initial Peak Width : 4 sec
Initial Tangent Height % : 10%
Monitor Noise : Before every run
Measurement Type : Peak Area
Initial Peak Reject Value : 200 counts (operator may adjust if necessary)
Report Unidentified Peaks : Yes
Report Missing Peaks : No
Normalize Results : No

Calibration Setup Address 44 *or 45* Channel Front *or Middle*
Calculation Type :Internal Standard
Number of Calibration Levels: 3
Curve Origin : Include
Curve Fit : Linear
Weighted Regression : (none)
Replicate Treatment : Keep Replicates Separate
Replicate Tolerance : Always add new replicates
Out-of-Tolerance Action : No Action
Out-of-Tolerance Action : No Action

Verification Setup Address 44 *or 45* Channel Front *or Middle*
Out-of-Tolerance Action : No Action

Peak Table Address *44 or 45* Channel Front (time windows may vary)

Reference Peak Time Windows: Width: 0.05 min. Retention Time 0.5%
Other Peaks Time Window : 0.05 min. Retention Time 0.5%

(retention times may vary)

| Ret.Time | Name | type | Level 1 | 2 | 3 |
|---|---|---|---|---|---|
| 1.160 | methanol | | .008 | .02 | .05 |
| 1.500 | ethanol | | .008 | .02 | .05 |
| 2.340 | IPA | | .008 | .02 | .05 |
| 2.070 | acetone | | .008 | .02 | .05 |
| 2.680 | n-propanol | STD | .045 | .045 | .045 |

Peak Table Address *44 or 45* Channel Middle
(retention times may vary)

| Ret.Time | Name | type | Level | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 1.160 | methanol | | | .008 | .02 | .05 |
| 1.500 | ethanol | | | .008 | .02 | .05 |
| 2.070 | acetone | | | .008 | .02 | .05 |
| 2.340 | IPA | | | .008 | .02 | .05 |
| 2.680 | n-propanol | STD | | .045 | .045 | .045 |

Time Event Table Address 44 *or 45* Channel Front *or Middle*
Time Events Table Empty
(Operator may choose to have events marked.)

Report Writer
A custom report writer can be utilized in the method.

4: Allow reagents and blood to equilibrate to room temperature before analysis.

5: Calibration Step - Calibrate the Varian 3800 gas chromatograph each time a new internal standard is prepared. With an appropriate liquid handler, deliver 0.2 ml of each of the calibrator solutions and 1.80 ml of the internal standard solution into separate headspace vials. Prepare three sets of four headspace vials that contain the calibrator solutions. Chromatograph the calibration standards on the Varian 3800 gas chromatograph to calibrate.

6: Quality Control and Verification Step - After calibration, the verification standard followed by a single water blank, and the commercial verification standard followed by a single water blank are chromatographed to check the system for resolution and precision. With an appropriate liquid handler, deliver 1.80 ml of internal standard and 0.20 mL of, the verification standard, the commercial verification standard, and water, respectively into separate headspace vials. Prepare the standards in duplicate. Chromatograph verification standards. Any measured component values exhibiting greater than 5% difference from targeted values will be rejected for that component. This step should be done once before case analysis on each day of case analysis. The operator may run other verification standards or blanks, in addition to the ones described above, at other times to insure system performance.

7: Blood Analysis - With an appropriate liquid handler, deliver 1.80 ml of internal standard and 0.200 ml of blood into a headspace vial. Prepare each blood sample in duplicate. Chromatograph each blood sample on each column.

8: Reporting - The alcohol or other volatile must be integrated at the expected retention time on both columns to be identified. The blood alcohol values shall be measured to the thousandths. The mean of the measured blood alcohol values is the blood alcohol concentration. Any blood alcohol values that exhibit greater than 5% difference as described by the equation; [({highest measured blood alcohol concentration minus lowest measured blood alcohol concentration} divided by lowest measured blood alcohol concentration) multiplied by 100], should be rerun. Blood samples where the highest measured alcohol value is 0.080 grams of alcohol or less per 100 milliliters of whole blood, do not need to be rerun if the other measured values are within 0.004 grams of alcohol per 100 milliliters of whole blood. For revocation reports, the blood alcohol concentration will be reported to the hundredths, and values between hundredths shall be reported to the next lower hundredth. Blood alcohol values equal to or less than 0.005 grams of alcohol per 100 milliliters of whole blood will be reported as zero. Clotted samples and samples where the analysts can only obtain a serum alcohol concentration or plasma alcohol concentration will be reported as an equivalent whole blood alcohol concentration by dividing the measured serum (plasma) alcohol concentration by 1.18 to compensate for the whole blood/serum (plasma) alcohol distribution ratio.

## References:

1. James C. Garriott (Editor), **Medicolegal Aspects of Alcohol**, 3rd Ed., 1996.

2. **LEAP's HS500 Static Headspace Autosampler Operating Manual**, LEAP Technologies.

3. **Varian 3800 Gas Chromatograph**, assorted manuals.

4. **Varian Star Chromatography Workstation**, assorted manuals.