Name of Procedure:

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 two Gas Chromatograph columns with different retention indexes, the identification of analytes can be determined and verified. By using 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 utilizing the retention times or relative retention times of standards chromatographed on both columns.

Apparatus and Chemicals Needed to Perform Procedure:

1: Headspace autosampler.
2: Gas chromatograph equipped with flame ionization detectors.
3: Restek BAC1 (Front) and BAC2 (Middle), 30m, 0.53 mm (i.d.) capillary columns.
4: Data Station.
5: Volumetric glassware:
Volumetric flasks - 100, 1000, and 2000 ml (TC) sizes.
Volumetric pipettes – 1, 5, 8, 10, 20, and 50 ml (TD) sizes.
6: Hamilton-Microlab 1000 plus, Hamilton 530B diluter/dispenser, or other appropriate liquid handler.
7: Headspace vials with sealing caps.
8: Crimper tool.
9: Ethanol, 200 proof USP Grade

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 shall 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 shall 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 0.010, 0.040, 0.080, 0.200, and 0.500 gram/100 ml calibrator solutions. Place the dates on the containers. Calibrator solutions shall be replaced every 3 months.

Example: Pipet 1, 4, 8, 20, and 50 ml respectively of the stock calibrator solution into 5 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 shall 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 shall 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: Gas chromatograph set up:

Method Parameters for alcohol determinations:

Initial Column Temperature (40^{o}C) Isothermal
Injector Temperature (200^{o}C)
Detector Temperature (200^{o}C)
Column Flow 12.5 ml/min (constant flow). (Nominal)
Split 1:2
Run Time 4:00-5:00 minutes
Detector make up flow: 18 ml/min
Detector H2 flow: 30 ml/min
Detector Air Flow: 300 ml/min

Method parameters for determination of other volatiles:

A temperature programmed method may be used to determine qualitatively other volatiles by utilizing the retention times or relative retention times of standards chromatographed on both columns.

2: Headspace Autosampler set up (The following parameters are recommended starting parameters. Autosampler parameters may be adjusted to permit improved performance.):

Incubation Temperature - 70°C
Incubation Time – 6:00 minutes
Agitator Speed – set to appropriate value
Extraction 1
Default Runtime 4:00-5:00 minutes
Syringe heater temperature 70°C
Sample Volume 1000 ul
A syringe bakeout of at least 5 °C above the injection temperature and a gas flush of at least 1.5 minutes will be performed between each injection.

3: Data Station:

The peak retention time windows will be set to the peak retention time +/- (0.05 minutes + 0.5% of the peak retention time).

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 gas chromatograph each time a new internal standard is prepared. With an appropriate liquid handler, deliver 0.20 ml of each of the calibrator solutions and 1.80 ml of the internal standard solution into separate headspace vials. Prepare each of the calibration solutions in duplicate. Chromatograph the calibration standards on the gas chromatograph to calibrate. The calibration curve will be fitted to a linear model with the origin included. The calibration curves for each analyte to be quantitated must show a correlation of determination (r^2) of 0.995 or greater.

The chromatographs produced from the calibration standards are reference materials and shall reviewed by another chemist and approved in the toxicology section object repository in FLAIRS with a file name beginning with "BACcal" and the date in yyyymmdd format with no space between them. (example: BACcal20080818).

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 verification standards in duplicate. Chromatograph and quantitate the verification standards. Any measured alcohol values exhibiting greater than 5% difference from targeted values will be rejected for that component. Due to its volatility, measured values of acetone that exhibit greater than 5% difference from the targeted value will be evaluated by the analyst. The water blanks must not show any identifiable amounts of methanol, ethanol, isopropanol, or acetone. This step shall be successfully completed before case analysis, and is applicable to cases injected within 24 hours of the injection of the first verification standard. The operator may run other verification standards or blanks, in addition to the ones described above, at other times to insure system performance. The chromatographs produced from the quality control samples are reference materials and shall reviewed by another chemist and approved in the toxicology section object repository of FLAIRS with a file name beginning with "BACver" and the date in yyyymmdd format with no space between them. (example: BACver20080818).

7: Blood Analysis - With an appropriate liquid handler, deliver 1.80 ml of internal standard and 0.20 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, or relative 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], shall 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 lower hundredth. Blood alcohol concentrations less than 0.010 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. Operation Manual(s) for the gas chromatograph.

3. Operation Manual(s) for the headspace autosampler.

4. Operation Manual(s) for the data system and applicable software.