FORENSIC SERVICES PROCEDURE MANUAL

BLOOD ALCOHOL ANALYSIS

QUANTITATIVE ALCOHOL ANALYSIS BY
HEADSPACE GAS CHROMATOGRAPHY

I. Equipment:

A. Hewlett Packard 5890 G.C.

B. Hewlett Packard 7694 Headspace Sampler

C. P.C. with Hewlett Packard ChemStation Version A.04.01

D. Micro Lab 500 Series, Auto Dilutor

E. Crimper, Hewlett Packard Cat. #9301-0720

II. Supplies:

A. Septa - Hewlett Packard - Cat. #9301-0976

B. Crimp Caps - Hewlett Packard - Cat. #9301-0721

C. 10 ml Headspace Vials - Hewlett Packard - Cat. #5182-0838

D. Whole Blood Control – ToxiChem- Cat. # 2930-14

E. Acetonitrile - Fisher Scientific

F. Methanol - Fisher Scientific

G. Acetone - Fisher Scientific

H. Isopropyl Alcohol - Fischer Scientific

I. Acetaldehyde - Fischer Scientific

J. .04, .10, .20, .30, Aqueous Ethanol Controls – College of American Pathologists- Cat. # STO11,17,18,19.

K. Mercuric Chloride - Fischer Scientific

L. Megabore INNOWAX 30 Meter Column - Hewlet Packard - Cat. # 19095N-123

II. Supplies (cont.)

M. Megabore DB-624 30 Meter Column - J & W Scientific - Cat. # 1251334

III. Reagent Preparation:

A. Preparation of Internal Standard Solution

1. Prepare 3% V/V acetonitrile stock solution from acetonitrile and deionized water - 30 ml Acetonitrile / liter of water + a pinch of mercuric chloride.

2. Prepare 0.012% W/V working internal standard solution - 5 ml stock solution / liter of water.

B. Preparation of Mixed standard

1. Acetaldehyde 0.25 ml, methanol 1.00 ml, acetone 0.25 ml, isopropyl alcohol 0.25 ml.

2. Mix with 1 liter of water + a pinch of mercuric chloride.

IV. Dilutor Preparation:

A. Check that there is enough internal standard for the analysis

B. Prime dilutor with internal standard (bubbles can be removed by first flushing the dilutor with acetone).

C. Set syringe volumes
1. Reagent = 2000 ul
2. Sample = 250 ul

V. Sample Preparation:

A. Label each sample vial .

B. Aspirate and dispense sample into vial. Prepare in duplicate.

C. Tightly crimp cap and septa onto vial.

D. Between each sample aspirate water (3x) and dispense into waste to rinse tubing. It is not necessary to rinse between duplicates.

VI. Standard, Blank, and Control Preparation:

A. Prepare .04, .10, .20, and .30 standards with aqueous standards using the same procedures as case samples.

B. Prepare blank with water using the same procedure as case samples.

C. Prepare control with known blood using the same procedures as case samples.

D. Prepare Mixed Standard using the same procedures as case samples.

VII. Calibration:

A. From "Sequence" menu click on "Load Sequence"

B. Highlight "calib.seq" and "OK".

C. From the "Sequence" menu click on "Edit Sequence Parameters".

D. Change the "Data File Subdirectory" to reflect the date of analysis and "OK".

E. Place aqueous calibrators (0.04, 0.100, 0.200, 0.300) in proper location on tray.

F. From the "RunControl" menu click on "Run Sequence".

G. From the "View" menu click on "Data Analysis".

H. From the "File" menu click on "Load Signal".

I. Highlight the first file (0.04 g/100 ml) and "OK".

J. From the "Calibration" menu click on "New Calibration Table" and click "OK" on the "Level One" box.

K. Click "yes" on the "Overwrite Existing Calibration Table" box.

L. Complete table by writing in "Name" (Ethanol or acetonitrile), "g/100 ml" (concentration) ,"ISTD" (ethanol-no, acetonitrile-yes), and "#" (be sure acetonitrile # matches ethanol # for each chromatogram.

VII. Calibration (cont.)

M. From the "File" menu click on "Load Signal", highlight the second file (0.100 g/100 ml) and "OK".

N. From the "Calibration" menu click on "Add Level" and "OK" the "Add Level 2" box. Fill in "Name" and "g/100 ml".

O. Repeat until all four levels are complete.

P. From the "View" menu click on "Method and Run Control". Click on "Method" and "Save Method" and "OK" "Overwrite Method". Enter "Recalibrate" in log.

VIII. Run preparation:

A. Place vials in sampler in the following order

1. Aqueous standards (0.04, 0.10, 0.20, 0.30).
2. Mixed standard
3. Blank
4. Blood control in duplicate
5. Case samples in duplicate
6. Blood control (Run a blood control at least every 10 samples).
7. Check standards (0.04, 0.10, 0.20, 0.30)

IX. Headspace and GC Parameters:

A. Carrier pressure - 0.25 bar

B. Vial pressure - 1.70 bar

C. GC Method - Bldalc1.M

D. Headspace Method - Bloodalc.hsm