

# 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 - Behring Diagnostics - Cat. #860161
- E. Acetonitrile - Fisher Scientific
- F. Methanol - Fisher Scientific
- G. Acetone - Fisher Scientific
- H. Isopropyl Alcohol - Fischer Scientific
- I. Acetaldehyde - Fischer Scientific
- J. .05, .10, .20, .30, Aqueous Ethanol Controls - Stephens Scientific -  
Cat. #4462-05 through 4462-30
- K. Mercuric Chloride - Fischer Scientific

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

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 .05, .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. Run preparation:

- A. Place vials in sampler in the following order
  - 1. Aqueous standards ( .05, .10, .20, .30 ) in duplicate.
  - 2. Mixed standard
  - 3. Blank
  - 4. Blood control in duplicate
  - 5. Case samples in duplicate
  - 6. Check standards ( .05, .10, .20, .30 )

VII. Headspace and GC Parameters:

- A. Carrier pressure - 0.25 bar
- B. Vial pressure - 1.70 bar
- C. GC Method - Bloodalc2.m
- D. Headspace Method - Bloodalc.hsm