Idaho State Police
Forensic Services
Toxicology Section

Section Four
Analysis of Alcohol and Common Volatile Solvents

4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles by Dual Column Headspace Gas Chromatography

4.1.1 BACKGROUND

Fermented beverages such as beer and wine have been known and used by humans since prehistoric times.[6] Ethanol abuse is often manifest in driving under the influence (DUI) problems, which is a worldwide concern. The National Highway Traffic Safety Administration (NHTSA) estimates that alcohol was involved in 41% of fatal automobile crashes and 7% of all crashes in 1995.[2] chronic alcoholism also contributes to ethanol related deaths. Ethanol consumed on a regular basis can lead to the development of alcoholic hepatitis which can progress into cirrhosis, liver failure, and death.[2,6,7] Chronic excessive ingestion of ethanol is directly associated with serious neurologic and mental disorders such as brain damage, memory loss, sleep disturbances and psychoses.[7] Alcohol is also involved in a high percentage of domestic disputes many of which result in injury and/or death.

Notwithstanding the public perception that ethanol is stimulatory, ethanol is classified as a *Central Nervous System Depressant.* Ethanol is a psychoactive drug that is similar in most respects to sedative-hypnotic compounds.[4] The first mental processes to be affected are those that depend on training and previous experience.[7] The individual's memory, concentration, and insight are dulled and subsequently lost. The person may become overly confident and exhibit uncontrolled mood swings and/or emotional outbursts.[7] The effects of ethanol and other central nervous system depressants are additive, resulting in more sedation and greater impairment of driving ability.[4]

Ethanol is rapidly and completely absorbed from the stomach, small intestine and colon. The mechanism of absorption is a simple diffusion process, that is, alcohol moving from a region of higher to a region of lower concentration.[2,4,6] Alcohol is soluble in both water and fat, a property that facilitates its diffusion through biological membranes.[4] The major amount of absorption takes place in the small intestine due to its large surface area, good blood supply and thin walled membrane. The time from the last drink to peak concentrations can range between 30 and 90 minutes, depending upon the individual's stomach contents.[4,7] Alcohol absorption is slowed by the presence of food in the stomach. The time period required for gastric emptying is a prime factor that contributes to the wide variety of absorption rates of ingested ethanol observed in different individuals and under different conditions.[2,7] Hence, the extent of absorption in the stomach and small intestine is a function of the amount of ethanol at that site, the vascularity of the site and the surface area in contract with the blood supply.[2] Other factors that affect the absorption of ethanol include the type of beverage, the alcohol content and any disease state that affects normal gastric function.[2]

Upon absorption, ethanol is distributed to all the water containing regions of the body. Within the blood there can be significant differences between arterial and venous blood depending upon the absorption status of the individual.[2] In the absorptive phase, the arterial blood ethanol concentration exceeds the venous blood ethanol concentration. Analysis of venous blood therefore, underestimates the brain alcohol concentration of the individual at this point. When absorption is complete there is little difference in ethanol concentration between and arterial and venous blood.[2]

90 to 98 percent of ethanol is completely oxidized in the liver by reacting with the cofactor nicotinamide adenine dinucleotide (NAD) facilitated by alcohol dehydrogenase to produce acetaldehyde. Acetaldehyde is then acted upon by aldehyde dehydrogenase to form acetic acid which goes onto form carbon dioxide and water (figure 1). The amount of ethanol oxidized per unit time is roughly proportional to body weight and probably to liver weight. The remaining (unoxidized) alcohol is excreted unchanged in urine, expired air, saliva and sweat. The average elimination rate of ethanol is 0.015 g/dL/hour from men and 0.018 g/dL/hour for women.[2] In addition to gender, chronic abuse, ethanol use combined with prescription drugs and certain genetic factors can also influence the elimination rate.[2,6,7]

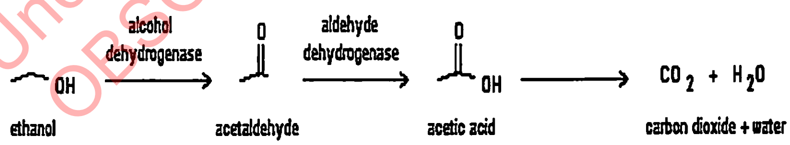

Figure 1. Metabolism of Ethanol.

Methanol (wood alcohol) causes relatively little intoxication compared to ethanol.[2,6] Its harmful affects are due to the direct result of its metabolism to formaldehyde (embalming fluid) and subsequently to formic acid. These metabolites lead to the destruction of neural cells, particularly the optic nerve, which can result in blindness.[2,6]

4.1.2 PRINCIPLE

This method describes the analysis of aqueous samples for the presence of volatile compounds including methanol, ethanol, acetaldehyde, acetone, isopropanol and related compounds, via a headspace sampling gas chromatographic method. Samples, controls and standards are sealed into vials that contain an aqueous 1-propanol internal standard solution and heated by the headspace analyzer. As described in Henry's Law, in a closed container at a given temperature, a direct (proportional) relationship exists between the amount of a volatile substance dissolved in a liquid and the amount of the volatile substance in the headspace vapor above the solution. An aliquot of the vapor is injected into a gas chromatograph (GC) in a dual column configuration. The GC serves to separate out the components of the solution as a function of their chemical properties. The separated components are identified on the basis of the retention time determined for each of the columns. Quantitation is accomplished through area percent data obtained from a flame ionization detector (FID). The quantitative result is based on a minimum of a three-point calibration curve, which uses the peak area ratio between the analyte and the internal standard.

4.1.3 EQUIPMENT

4.1.3.1 Perkin Elmer Auto System XL Gas Chromatograph (GC)

4.1.3.2 <u>Columns</u>

4.1.3.2.1 Restek Rtx®-BAC1 (#18003: 30 meter X 0.32mm inner diameter (ID), 1.8µm film thickness (FT)) or equivalent column

4.1.3.2.2 Restek Rtx®-BAC2 (#18002: 30 meter X 0.32mm ID, 1.2 µm film thickness (FT)) or equivalent column

4.1.3.3 Perkin Elmer HS-40 or HS-110 Headspace Autosampler (figures 2 and 3)

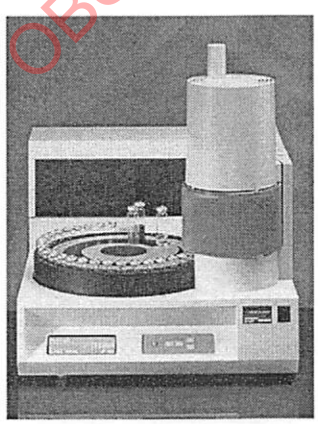

Figure 2. HS-40

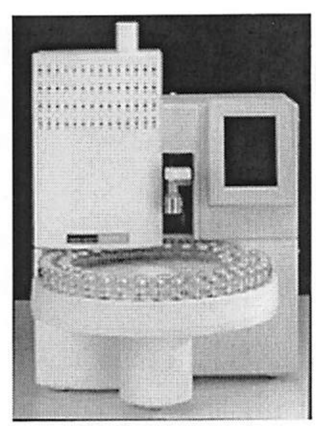

Figure 2. HS-110

4.1.3.4 PE Workstation Software, TotalChrom Version 6.2.0 or more recent version/upgrade.

4.1.3.5 Hand Crimper (P-E B003-8134 or equivalent)

4.1.3.6 Hamilton MICROLAB 503A or equivalent semi-automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250µL and 2000µL, respectively.

4.1.3.7 Glassware

4.1.3.7.1 GC-Headspace vials (P-E B010-4236 or equivalent)

4.1.3.7.2 Safety Closures {PTBE septa, crimp caps and star springs} (P-E BO10-4240 or equivalent)

4.1.4 CONTROLS AND CALIBRATORS

4.1.4.1 Whole Blood Ethanol Control (LiquiSP$_x$™ or equivalent).

4.1.4.2 Aqueous Ethanol Standards (g/100mL)
0.025, 0.05, 0.08, 0.10, 0.20, 0.30, and 0.40 (Cerilliant or equivalent)

4.1.4.3 Multicomponent alcohol Calibration Kit (Cerilliant #A-054 or equivalent)

4.1.5 REAGENTS

4.1.5.1 1-Propanol (Acros/Fisher Scientific # 23207-0010, #A996-1 or equivalent)

4.1.5.2 Acetone (Fisher #A929-1 or equivalent)

4.1.5.3 Acetaldehyde (Fisher #01004-250 or equivalent)

4.1.5.4 Isopropanol (2-Propanol) (Fisher #A416-500 or equivalent)

4.1.5.5 Methanol (Fisher #A454-1 or equivalent)

4.1.5.6 Ammonium Sulfate (Fisher #A702-500 or equivalent)

4.1.5.7 Sodium Fluoride (Fisher #S299-500 or equivalent)

4.1.6 SAFETY CONCERNS

4.1.6.1 Blood samples should be processed according to safety guidelines in the *Chemical Hygiene and Safety Manual*.

4.1.7 REAGENT PREPARATION

Record the preparation of all reagents on reagent log.

4.1.7.1 Internal Standard Solution
{0.03g/dL 1-propanol in 1.0M $(NH_4)_2SO_4$}

4.1.7.1.1 1.0M $(NH_4)_2SO_4$
Dissolve 132.14g $(NH_4)_2SO_4$ in distilled water. Dilute to 1L.

4.1.7.1.2 0.03g/dL 1-propanol in 1.0M $(NH_4)_2SO_4$

- Add approximately 800mL of 1.0M $(NH_4)_2SO_4$ to a 1000mL volumetric flask.
- Add 1g sodium fluoride *{optional}*.
- Add 375µL 1-propanol. QS to 1000mL.

4.1.7.2 Volatile Standard Mix Solution

4.1.7.2.1 Add approximately 200 mL of DI water to a 250-mL volumetric flask.

4.1.7.2.2 Add the following volatiles, as indicated:

- 100 µL acetaldehyde
- 100 µL acetone
- 500 µL methanol
- 500 µL isopropanol
- 500 µL ethanol

4.1.7.2.3 QS to 250-mL.

4.1.8 ANALYSIS PROCEDURE

4.1.8.1 General

4.1.8.1.1 Bring calibrators, controls, internal standard and samples to room temperature.

4.1.8.1.2 Gather necessary vials, closures and ancillary supplies in or near laminar flow hood.

4.1.8.1.3 Sample preparation should take place in a laminar flow hood.

4.1.8.2 Quality Control

4.1.8.2.1 Ethanol calibration standards must be run prior to the analysis of each batch of samples. A minimum of three points of calibration should be established.

4.1.8.2.2 An internal standard blank should follow the last ethanol calibrator.

4.1.8.2.3 A blood or aqueous control sample must be run after every 10 case samples. A minimum of two blood controls must be run per batch of samples.

4.1.8.2.4 Refer to package insert for manufacturer blood control ranges.

4.1.8.2.5 Values obtained from aqueous control and whole blood control samples must agree ± 10% of their target values.

4.1.8.2.6 Periodically run either the Volatile Standard Mix Solution or the Multicomponent Alcohol Calibration Kit solution to determine and monitor the retention of other volatiles of interest.

4.1.8.2.7 Record values for blood control samples in B*atch Analysis QC log.*

4.1.8.2.8 On a monthly basis calculate the mean, standard deviation, relative standard deviation (CV%) and percent accuracy of the control samples. The data will be used to generate a mean quality control chart.

4.1.8.3 Pipetter/Dilutor Set-up

4.1.8.3.1 Switch on power.

4.1.8.3.2 Display will inquire as to the sizes of installed syringes. Select the correct size for sample syringe [right] and reagent syringe [left].

4.1.8.3.3 Scroll down to volume option. Select 250µL for sample syringe [right] and 2000µL for reagent syringe [left].

4.1.8.3.4 Scroll down to speed option. Verify that syringe speed is on desired setting.

4.1.8.3.5 Prime the fluid path. Continue priming until no bubbles are observed.

4.1.8.4 Preparation of Blanks, Blood Control and Mixed Standard

4.1.8.4.1 Water Blank

4.1.8.4.1.1 Label test vial with *water blank*.

4.1.8.4.1.2 Add 2000µL DI water to labeled test tube.

4.1.8.4.1.3 Seal immediately with crimp cap as illustrated in figure 4.

4.1.8.4.2 Internal Standard Blank

4.1.8.4.2.1 Label test vial with *ISTD blank*.

4.1.8.4.2.2 Use Pipetter/Dilutor to dispense 2000µL of internal standard (ISTD) into labeled headspace vial.

4.1.8.4.2.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.3 Blood Control

4.1.8.4.3.1 Label two headspace vials for *blood control 1 and 2*.

4.1.8.4.3.2 Use Pipetter/Dilutor to dispense 250µL of blood control and 2000µL of internal standard (ISTD) into each labeled headspace vial.

4.1.8.4.3.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.4 Aqueous Controls

4.1.8.4.4.1 Label appropriate number of headspace vials for *aqueous controls (1, 2,...)*.

4.1.8.4.3.2 Use Pipetter/Dilutor to dispense 250μL of aqueous control and 2000μL of internal standard (ISTD) into each labeled headspace vial.

4.1.8.4.3.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.5 Mixed Other Volatiles Solution

4.1.8.4.5.1 Label test vial with *mixed volatiles.*

4.1.8.4.5.2 Use Pipetter/Dilutor to dispense 250μL of mixed volatile solution and 2000μL of internal standard (ISTD) into labeled headspace vial.

4.1.8.4.5.3 Seal **immediately** with crimp cap as illustrated in figure 4.

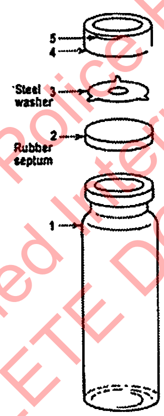

Figure 4. Crimp cap assembly

4.1.8.5 Preparation Calibration Standards

4.1.8.5.1 Label vials for standards in duplicate.

4.1.8.5.2 Use Pipetter/Dilutor to dispense 250μL of appropriate ethanol concentration and 2000μL of internal standard (ISTD) into each labeled headspace vial.

4.1.8.5.3 Seal **immediately** with crimp cap.

4.1.8.5.4 Establish ethanol calibration plot with a minimum of three calibration points.

4.1.8.6 Initial Processing of Specimens

4.1.8.6.1 Open the sample submittal kit and remove the specimen's inner compartment. After inspecting and noting the condition of seals, open inner compartment (plastic tray or biohazard bag) and place laboratory number on each blood/urine/vitreous humor specimen.

4.1.8.6.2 When two blood/fluid samples are present, the samples should be labeled "A" and "B" or equivalent. Utilize sample "A" for analysis unless it contains insufficient sample.

4.1.8.7 Preparation of Samples for Analysis

4.1.8.7.1 Label two headspace vials with the laboratory number without the prefix.

4.1.8.7.2 Place one of the sample tubes or urine specimen bottle on tube rocker for at least two minutes.

4.1.8.8 Addition of blood, urine or vitreous humor sample to headspace vials.

4.1.8.8.1 Use Pipetter/Dilutor dispense 250µL of sample and 2000µL of internal standard (ISTD) to a labeled headspace vial.

4.1.8.8.2 Seal headspace vials **immediately** with crimp caps as illustrated in figure 4.

4.1.8.9 Preparation for Run

4.1.8.9.1 Open **Sequence Editor**

4.1.8.9.2 Into Sequence log table, enter the sample case numbers, ethanol standards, other volatiles mix, blanks and controls.

4.1.8.9.3 Load samples, calibration standards, blank and controls into the carousel of the headspace sampler as noted in the sequence table.

4.1.8.9.4 Active headspace sampler

- Click on the **Setup** button to open the setup instrument dialog box.

- Select sequence as the setup type, and select the desired sequence file.
- On **Setup Instrument** dialog box, designate starting and ending row.
- Verify that the paths for raw and result data files specified in the sequence indicate the desired destinations.
- Select OK in the **Setup Instrument** dialog box to initialize the instrument.

4.1.8.10 Gas Chromatography Parameters

4.1.8.10.1 Refer to instrument METHOD printout for oven program and zone temperatures. Temperature program must provide for baseline separation of volatile compounds of interest as indicated by analysis of multicomponent mixtures.

4.1.8.11 Calibration

4.1.8.11.1 Ethanol calibrators should be analyzed in order of increasing concentration.

4.1.8.11.2 The least squares line resulting from the analysis of the ethanol calibrators must have a coefficient of correlation of ≥0.999.

4.1.8.12 Acceptance Criteria

4.1.8.12.1 **Accuracy**

4.1.8.12.1.1 Qualitative

The presence of ethanol can be established if there are no significant differences in the retention time between sample and standards. The relative retention times for a specimen must be within ± 0.10 minutes of the relative retention time for the compound in question. This rejection criterion should be designated in the TotalChrom analysis method.

4.1.8.12.1.2 Quantitative

The quantitative results for a batch of samples can be accepted if the values obtained for control samples fall within 10% of their target value range.

4.1.8.12.2 **Precision**

The results obtained from duplicate analysis must agree within 0.015g/100mL. If this precision requirement is not met, the sample is reanalyzed.

4.1.8.13 Reporting of Results

4.1.8.13.1 **Blood**

Samples are quantitated to three significant figures. Report truncated mean value, of grams of ethanol per 100cc of whole blood, to two significant figures.

4.1.8.13.2 **Urine**

Samples are quantitated to three significant figures. Result obtained from blood alcohol curve should be multiplied by 0.67. Report truncated mean value, as grams of ethanol per 67 mL of urine, to two significant figures. A warning statement such as *Urine results may be of questionable value*, must be included in the report.

4.1.8.13.3 **Vitreous Humor**

Samples are quantitated to three significant figures. Report truncated mean value, as grams of ethanol per 100mL of vitreous humor, to two significant figures.

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4.1.9 QUALITY ASSURANCE

4.1.9.1 Blood or vitreous samples are to be refrigerated while at the laboratory. Urine samples can be either refrigerated or frozen.

4.1.9.2 Refer to toxicology manual section 5.1 for pipette calibration options.

4.1.9.3 Refer to toxicology manual section 5.2 for balance calibration requirements.

4.1.9.4 Refer to toxicology manual section 5.3.2 for GC-HS maintenance schedule.

4.1.9.5 Blood calibrators should be ordered prior to the current supply running out. This will allow for the analysis of new lots against existing calibrators.

4.1.10 REFERENCES

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4.1.10.8 Idaho Administration Code, IDAPA 11.03.01, Rules Governing Alcohol Testing.

4.1.10.9 Christmore, D.S., Kelly, R.C. and Doshier, L.A. *Improved Recovery and Stability of Ethanol in Automated Headspace Analysis,* J. Forensic Sci. 29(4): 1038-1044; 1984.

4.1.10.10 Restek Applications Note #59598, Dual-Column Confirmational GC Analysis of Blood Alcohols Using the Rtx®-BAC1 and Rtx®-BAC2 Columns Optimized for the Perkin-Elmer HS-40 Headspace Autosampler, 1999.

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Blood Volatiles Determination
4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles by Dual Column Headspace Gas Chromatography

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0	10/01	Original Issue

Approval

Technical Leader: [signature] S C Williamson **Date:** 10-31-01

Issuance

QC Manager: [signature] Rick D. Groff **Date:** 1-24-02