

Idaho State Police

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4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles in Blood, Vitreous Humor and Urine by Dual Column Headspace Gas Chromatography

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Section Four

Analysis of Alcohol and Common Volatile Solvents

4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles in Blood, Vitreous Humor and Urine by Dual Column Headspace Gas Chromatography

4.1.1 BACKGROUND

Humans have consumed fermented beverages such as beer and wine since prehistoric times.⁶ Ethanol abuse is often manifest in driving under the influence (DUI), a problem that plagues every country. The National Highway Traffic Safety Administration (NHTSA) reported that the proportion of driver involved in fatal crashes who had a BAC of 0.08 or above decreased from 35% in 1982 to 20% in 1997 and leveled off thereafter.¹⁷ Changes in alcohol laws and demographic changes are thought to be responsible for this positive trend. 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 a common 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 a greater degree of impairment in 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 moves from a region of higher concentration 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

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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 contact with the blood supply.² Other factors that affect the absorption of ethanol include the type of beverage, the alcohol content, the rate of consumption and any disease state that affects normal gastric function or blood flow.²

Upon absorption, ethanol is distributed to all the water containing regions of the body. Within the blood system 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 arterial and venous blood.²

Ninety to ninety-eight 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 on to 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, prescription drugs and certain genetic factors can also influence the elimination rate.^{2,6,7}

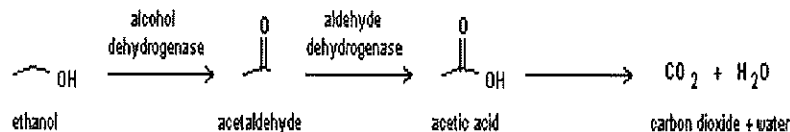


Figure 1. Metabolism of Ethanol.

Other commonly encountered alcohols such as methanol and isopropanol produce central nervous system (CNS) depressant effects but vary significantly in the degree. Methanol (wood alcohol) causes relatively little intoxication compared to ethanol.^{2,6} Its harmful effects 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} The formic acid leads to metabolic

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acidosis. Isopropanol (rubbing alcohol) is more toxic than ethanol with more prominent gastritis that includes pain, nausea, vomiting and hemorrhage.¹¹ Isopropanol is metabolized to acetone.

Toluene and acetone are commonly encountered in subjects that intentionally inhale ("huff") products including paint and contact adhesives to achieve effects akin to those of ethanol. The principal metabolite of toluene is benzoic acid. Chronic abuse of toluene and/or acetone can lead to organ and CNS problems that may result in permanent damage.¹² Elevated endogenous acetone may be detected in the blood and urine of a diabetic or fasting individual. Acetone is metabolized to acetate and formate.

The analysis of ethanol and other volatiles in samples of blood, vitreous humor and urine is accomplished with a gas chromatograph (GC) which uses a headspace analyzer for sample introduction. An aliquot of sample is placed into a headspace vial along with an aqueous 1-propanol internal standard. The sample vials are then sealed and heated in a 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 the GC in a dual column configuration. The GC serves to separate out the components of the solution as a function of their chemical properties. Separated components are detected by a flame ionization detector (FID). The qualitative identification of ethanol and other common volatiles is based on the retention time determined, relative to the 1-propanol internal standard, for each of the columns. This method also provides for a quantitative determination for ethanol. The quantitative result is based on a minimum of a three-point calibration curve, which uses the peak area ratio between ethanol and the 1-propanol internal standard.

4.1.2 SCOPE

This method describes the Idaho State Police Forensic Services (ISP-FS) procedure for the analysis of blood, vitreous humor or urine for the presence of volatile compounds. This method provides for the quantitative analysis of ethanol as well as the qualitative analysis of methanol, acetaldehyde, acetone, isopropanol, toluene and related compounds, via a headspace sampling gas chromatographic method. The oven temperature program for Gas Chromatograph must provide for baseline separation of volatile compounds of interest as indicated by analysis of multicomponent mixtures. IDAPA 11.03.01 (Rules Governing Alcohol Testing) requires that blood ethanol results (BAC) for legal purposes (in living subjects) be reported in units of *grams of alcohol per one hundred (100) cubic centimeters of whole blood*.⁸ For this reason, serum is not an appropriate sample under IDAPA 11.03.01. If serum is analyzed a qualifier advising that the sample does not comply with IDAPA 11.03.01 must be placed on the analysis report. Urine analysis is allowed under IDAPA 11.03.01 but due to the questionable validity of obtained results, a

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qualifier must be added to the analysis report.⁸ Urine ethanol result (UAC) issues include correlation with blood alcohol (UAC/BAC ratio) and endogenous production of ethanol.⁹⁻¹¹

4.1.3 EQUIPMENT

4.1.3.1 Agilent 7890A Gas Chromatograph (GC) configured with a Flame Ionization Detector (FID) (Figure 2).

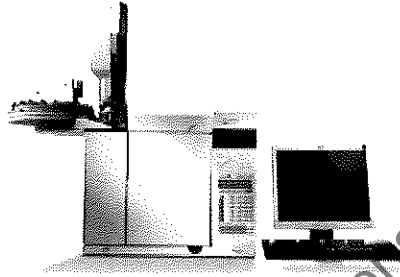


Figure 2. Gas Chromatograph

4.1.3.2 Agilent G1888 Headspace Sampler (Figure 3).

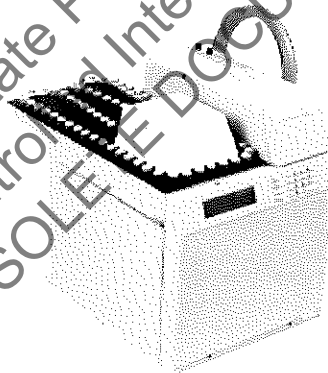


Figure 3. Headspace Analyzer

4.1.3.3 Columns

4.1.3.3.1 Restek Rtx[®]-BAC1 (#18003: 30 meter X 0.32mm inner diameter (ID), 1.8 μ m film thickness (FT) or equivalent column)

4.1.3.3.2 Restek Rtx[®]-BAC2 (#18002: 30 meter X 0.32mm ID, 1.2 μ m FT or equivalent column)

4.1.3.4 Headspace (HS) vials and Closures

4.1.3.5 Hand Crimper or Bench Top Crimper

4.1.3.6 Semi-Automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250 μ L and 2000 μ L, respectively.

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4.1.4 REAGENTS

When available, a certificate of analysis should be obtained and centrally stored.

- 4.1.4.1 Distilled/Deionized water (free from volatiles of interest)
- 4.1.4.2 1-Propanol/n-Propanol ($\geq 99\%$)
- 4.1.4.3 Acetone ($\geq 99\%$)
- 4.1.4.4 Acetaldehyde ($\geq 99\%$)
- 4.1.4.5 Isopropanol/2-Propanol ($\geq 99\%$)
- 4.1.4.6 Methanol ($\geq 99\%$)
- 4.1.4.7 Toluene ($\geq 99\%$)
- 4.1.4.8 Ammonium Sulfate (Certified ACS Grade)

4.1.5 QUALITY ASSURANCE MATERIAL**4.1.5.1 Ethanol Aqueous Reference Material**

4.1.5.1.1 Aqueous ethanol reference material used to establish the calibration curve/table or to prepare ethanol aqueous controls can be obtained through Cerilliant, EM Science, NIST or other appropriate vendor.

4.1.5.1.2 Whenever possible, the source (vendor or lot number) of reference material used for a particular calibrator must be different from that used to prepare a particular aqueous control samples. For instance, if a 0.08g/100mL *control* is prepared from a particular lot of Cerilliant solution, either a different lot number from Cerilliant or another vendor should be used to prepare a 0.08g/100mL *calibrator*.

4.1.5.2 Ethanol or Multicomponent Whole Blood Reference Material

4.1.5.2.1 Whole blood containing ethanol, with or without other volatiles of interest, used to prepare matrix controls can be obtained through BIORAD or other appropriate vendor.

4.1.5.2.2 A minimum of two ethanol whole blood control levels must be available, each falling within the following ranges:

Level	Ethanol Range (g/100mL)
1 - Low	0.04 – 0.09
2 - High	0.13 – 0.40

4.1.5.2.3 A whole blood control containing ethanol with other volatiles can serve as a multicomponent control if the

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GC oven temperature program provides for baseline separation of all components.

4.1.5.3 Multicomponent Volatile Aqueous Solutions

Multicomponent solutions may be purchased or prepared as indicated below.

4.1.5.3.1 **Commercially Obtained Multicomponent Solution**

4.1.5.3.1.1 Solution may include acetone, ethanol, methanol and isopropanol reference materials and/other commonly abused volatiles.

4.1.5.3.1.2 When the solution contains quantitative amounts of volatiles other than ethanol, this method utilizes/analyzes them qualitatively.

4.1.5.3.1.3 When the multicomponent solution contains quantitative amounts of ethanol, it may simultaneously serve as an aqueous ethanol control if the GC oven temperature program provides for baseline separation of all components.

4.1.5.3.2 **Prepared Mixed Volatile Solution**

4.1.5.3.2.1 Add approximately 200mL of DI water to a 250mL volumetric flask. Add **one or more** of the following volatiles, as needed for the qualitative identification of volatiles:

Compound	Volume
Acetaldehyde	100 μ L
Acetone	100 μ L
Ethanol	100 μ L
Ethyl Acetate	100 μ L
Methanol	500 μ L
Isopropanol	500 μ L
Toluene	50 μ L

QS to 250-mL. Record preparation on reagent log. Solution is stable for 1 year when stored under refrigeration.

4.1.5.3.2.2 Additional volatiles of interest may be added to the mixed volatile solution.

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The GC oven temperature conditions must provide for baseline separation from other components in the mixture.

4.1.5.4 Internal Standard Solution

0.03g/dL 1-propanol in 1.0M Ammonium Sulfate

4.1.5.4.1 Add approximately 800mL of DI water to a 1L volumetric flask. Add 132.14g $(\text{NH}_4)_2\text{SO}_4$ and mix to dissolve. Add 375 μL 1-propanol. QS to 1L with distilled water.

4.1.5.4.2 Record preparation on reagent log. Solution is stable for 1 month when stored at room temperature. Other volumes of internal standard may be prepared as needed.

4.1.6 **SAFETY CONCERNS**

Blood samples must be processed and chemicals handled according to safety guidelines in the *Idaho State Police Forensic Services Health and Safety Manual*.

4.1.7 **QUALITY ASSURANCE**

4.1.7.1 General

4.1.7.1.1 While at the laboratory blood or vitreous humor samples are to be stored under refrigeration. Urine samples can be either refrigerated or frozen. Urine samples must be frozen for long-term storage.

4.1.7.1.2 The syringes on the Pipetter/Dilutor must be checked for accuracy and precision. Refer to toxicology manual section 5.1 for pipette intermediate check and calibration requirements and options.

4.1.7.1.3 Refer to toxicology manual section 5.2 for balance intermediate check and calibration requirements.

Note: Balances properly monitored by drug discipline analysts fulfills quality assurance requirements. Additional check need not be performed.

4.1.7.1.4 Refer to manufacturer manuals for recommended instrument maintenance and troubleshooting measures.

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4.1.7.1.5 Current source and lot number of controls and reference material must be maintained on spreadsheet forms.

4.1.7.2 Calibration Curve/Table Requirements

4.1.7.2.1 A minimum of three ethanol aqueous reference solutions must be used to establish a calibration curve.

4.1.7.2.1.1 The minimum low calibrator may be either 0.020, 0.025 or 0.05g/100mL.

4.1.7.2.1.2 The highest calibrator may be either a 0.40 or 0.50g/100mL.

4.1.7.2.1.3 All sample and control values must have a calibrator greater than or equal to their mean value.

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

4.1.7.2.3 The least squares line resulting from the analysis of the ethanol calibrators must have a coefficient of correlation of ≥ 0.998 .

4.1.7.2.4 Each ethanol calibrator may have more than one replicate.

4.1.7.2.4.1 In the sequence table, on the **Update RF** column select "replace" from the pull-down for each of the first set of calibrators. If a second set of calibrators is run, select "average".

4.1.7.2.5 Regardless of whether calibration reference materials are run singularly or in duplicate, if one or more of the replicates are not usable the remaining data can be used to establish the response factor provided:

- ✓ There is a minimum of three remaining distinct points.
- ✓ Regression requirements are met.

4.1.7.2.6 A calibration curve/table is valid for 14 days provided all values for required controls fall within acceptable ranges and the same preparation of internal standard solution used for the calibration run is available.

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- 4.1.7.2.7 Once established, analysts not involved in establishing the calibration curve/table may use the established calibration.
- 4.1.7.2.8 An analysis run may include case samples prepared by more than one analyst.
- 4.1.7.3 Per Analysis Run Control Requirements
- 4.1.7.3.1 **Calibration Run**
- 4.1.7.3.1.1 An internal standard blank should follow the last (highest) ethanol calibrator.
- 4.1.7.3.1.2 A water blank may be included in each calibration run.
- 4.1.7.3.1.3 For up to 10 samples (20 vials), an analysis run must include either a high or low blood control in duplicate before proceeding with additional samples.
- 4.1.7.3.1.4 For analysis run consisting of more than 10 samples (20 vials), a minimum of one blood or aqueous control must be run with each additional 10 samples (20 vials).
- 4.1.7.3.1.5 A blood or aqueous control containing ethanol, with or without other volatile substances, meets the "per run" requirement.
- 4.1.7.3.1.6 Each calibration run must include either an aqueous or blood multicomponent volatile mix.
- 4.1.7.3.1.7 A commercially obtained quantitative multicomponent volatile mix may be used as both an aqueous ethanol control and a multicomponent mixture.
- 4.1.7.3.2 **Additional Runs with Existing Calibration Table**
- 4.1.7.3.2.1 The same batch of internal standard used in the calibration run must be used for additional runs within the two-week period.

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- 4.1.7.3.2.2 An internal standard blank should follow the analysis of the high blood control.
- 4.1.7.3.2.3 The analysis run must include both high and low blood and/or aqueous controls in duplicate. At least one set of duplicates must be blood controls.
- 4.1.7.3.2.4 When the analysis exceeds 10 samples (20 vials), a minimum of one blood or aqueous control must be run with each additional 10 samples (20 vials).
- 4.1.7.3.2.5 A blood or aqueous control containing ethanol, with or without other volatile substances, meets the "per run" requirement.
- 4.1.7.3.2.6 Additional aqueous controls can be included at the discretion of the analyst.

4.1.7.4 Uncertainty of Measurement

4.1.7.4.1 Due to the uncertainty of measurement associated with any quantitative measurement, the standard deviation of blood control values and proficiency testing data, must be continually monitored. Blood control values must be entered onto I:\ drive uncertainty spreadsheet after each analysis run.

4.1.7.4.2 Uncertainty value will be two standard deviations as calculated after each analysis run by uncertainty spreadsheet.

4.1.7.4.3 The uncertainty value will be subtracted from the mean ethanol value for each sample. The current uncertainty value may be entered into the instrument casefile tab of the "volatiles" EXCEL spreadsheet prior to each analysis run. If used, the spreadsheet will calculate the adjusted ethanol concentration value and will be reflected on the casefile print-out by the MACRO.

4.1.8 PRE-RUN COLLECTION KIT PROCESSING

4.1.8.1 Collection Kit Description and Labeling

4.1.8.1.1 Note sample container description on *Volatiles Analysis Coversheet*.

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- 4.1.8.1.2 Inspect inner seals on specimen container and note their condition on *Volatiles Analysis Coversheet*.
- 4.1.8.1.3 When the version of the toxicology submittal form requires it, note condition of seals and initial.
- 4.1.8.1.4 Laboratory number must be placed on each sample container.
- 4.1.8.1.5 When more than one sample is present, label samples "A", "B", etc., or comparable. Note container type(s) on analysis coversheet.
- 4.1.8.2 Case Sample and Collection Container Evaluation
- 4.1.8.2.1 Note sample type (blood, urine, or vitreous humor) on *Volatiles Analysis Coversheet*.
- 4.1.8.2.2 When the sample is blood or urine, the sample container (tube or bottle) and contents must be examined to determine compliance with Idaho Administrative Code, IDAPA 11.03.01.⁸
- 4.1.8.2.3 IDAPA 11.03.01 does not apply to specimens collected from deceased individuals; however, a qualifier statement, advising how the integrity of the sample is adversely affected, may be added to the report as described in section 4.1.12.5.2
- 4.1.8.2.4 **Blood Specimen Container Evaluation**
- 4.1.8.2.4.1 IDAPA requires that blood specimens be collected in a container, which contains *ten (10) milligrams of sodium fluoride per cubic centimeter of blood plus an appropriate anticoagulant*.
- 4.1.8.2.4.2 The containers provided in ISP-FS kits comply with these requirements. It must, however, not be assumed that an ISP-FS kit contains the container(s) it was supplied with.
- 4.1.8.2.4.3 Non-ISP-containers must be evaluated as to compliance. Information from the manufacturer of the container will indicate sodium fluoride concentration

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as well as the presence of an appropriate anticoagulant.

4.1.8.2.4.4 Note specimen container compliance on *Volatiles Analysis Coversheet*.

4.1.8.2.5 **Urine Specimen Container Evaluation**

4.1.8.2.5.1 IDAPA requires that urine specimens be collected in clean, dry containers.

4.1.8.2.5.2 The containers provided in ISP-FS kits comply with these requirements. It must, however, not be assumed that an ISP-FS kit contains the container it was supplied with.

4.1.8.2.5.3 Note type of specimen container on *Volatiles Analysis Coversheet*.

4.1.8.2.6 **Blood Specimen Evaluation**

4.1.8.2.6.1 IDAPA 11.03.01 requires blood to be reported as grams of alcohol per 100cc of whole blood. When it is the analyst's opinion that the intended blood sample is serum or otherwise questionable, or with a questionable urine sample, the analyst has the following options.

4.1.8.2.6.2 Option One

The sample is not analyzed. A comment "Sample/specimen unsuitable for analysis" is placed on the analysis report.

4.1.8.2.6.3 Option Two

The sample is analyzed in accordance with this Analytical Method. A qualifier is placed on the analysis report, "The sample does not comply with IDAPA 11.03.01". This option may only be used for samples from non-deceased subjects.

4.1.8.2.6.4 Option Three

The sample is analyzed for ethanol and other volatiles according to Analytical Method 4.2, Analysis of Solutions

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Containing Ethanol and Common Volatiles. The report, therefore, will make no mention of the sample having a biological origin.

4.1.9 PRE-RUN PIPETTER/DILUTOR SET-UP

- 4.1.9.1 Switch on power.
- 4.1.9.2 Display will inquire as to the sizes of installed syringes. Select the correct size for sample syringe [right] and reagent syringe [left].
- 4.1.9.3 Scroll down to volume option. Select 250 μ L for sample syringe [right] and 2000 μ L for reagent syringe [left].
- 4.1.9.4 Scroll down to speed option. Verify that syringe speed is on desired setting.
- 4.1.9.5 Prime the fluid path. Continue priming until no bubbles are observed.

4.1.10 ANALYSIS PROCEDURE

4.1.10.1 General

- 4.1.10.1.1 Bring calibrators, controls, internal standard and samples to room temperature.
- 4.1.10.1.2 Label headspace vials for case samples, calibrators and controls.
- 4.1.10.1.3 Sample preparation must take place in a laminar flow hood or biological safety cabinet.
- 4.1.10.1.4 Place blood sample container on rocker for a minimum of two minutes.
- 4.1.10.1.5 If blood sample appears to be coagulated, the sample may require homogenization in a tissue grinder, or equivalent.
- 4.1.10.1.6 Lot numbers for calibrators and controls must be recorded on task sheet, in sequence, and/or solution preparation log.

4.1.10.2 Headspace Vial Preparation

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- 4.1.10.2.1 Use Pipetter/Dilutor dispense 250 μ L of case samples, controls and calibrator solutions along with 2000 μ L of internal standard (ISTD) into labeled headspace vial.
- 4.1.10.2.2 Use Pipetter/Dilutor to dispense 2000 μ L of internal standard (ISTD) into labeled headspace vial.
- 4.1.10.2.3 Seal headspace vials **immediately** with crimp caps.

4.1.10.3 Instrument Run Preparation

- 4.1.10.3.1 Open **Sequence Table**. It is recommended that each analyst prepare and use their own sequence. This reduces the possibility of the sequence being modified without their knowledge.
- 4.1.10.3.2 Into Sequence log table, enter the sample case numbers, ethanol calibrators, other volatiles mix, blanks and controls.
- 4.1.10.3.3 Load samples, calibrators, blank and controls onto the headspace sampler carousel rack as noted in the sequence table.
- 4.1.10.3.4 The sequence information should be verified prior to starting the instrument.

4.1.10.4 Instrument Parameters

- 4.1.10.4.1 Refer to instrument METHOD printouts for gas chromatograph and headspace analyzer analysis parameters.
- 4.1.10.4.2 The method must be set up such that all samples (casework, calibrators, and controls) are quantitated to a minimum of three decimal places (0.000).
- 4.1.10.4.3 Analysis method printouts must be stored centrally (hardcopy and/or electronically).

4.1.11 ACCEPTANCE CRITERIA

4.1.11.1 Qualitative Accuracy Criteria

The qualitative presence of ethanol, or other volatile substances, can be established if the relative retention time (RRT) for a specimen is within ± 0.10 minutes of the RRT of the reference compound in question. This criterion should be designated in the instrument's data station analysis method.

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4.1.11.2 Quantitative Accuracy Criteria

4.1.11.2.1 If desired, a MACRO, on the instrument's computer can enter the ethanol concentrations for the duplicate case samples, for each column, on the case sample page of a spreadsheet. The spreadsheet must be formatted such that mean ethanol values are calculated using a minimum of three decimal places, the current uncertainty value is subtracted, and the adjusted value is truncated to two decimal places for reporting.

4.1.11.2.2 If an instrument MACRO is not used, a copy of the I:\ drive "Volatiles Determination Casefile Worksheet" (or equivalent) can be used. The casefile TAB is used to calculate case sample ethanol values. The calibrator and control TABS are used to calculate the mean values for calibrator and control samples.

4.1.11.2.3 The quantitative ethanol results for a batch of samples can be accepted if the values obtained for control samples fall within $\pm 10\%$ of target value. Target values are determined as described in sections 4.1.14.1.5 and 4.1.14.1.6.

4.1.11.3 Column Precision Criteria

4.1.11.3.1 If desired, a MACRO, on the instrument's computer, can enter the ethanol concentrations for the duplicate samples, for each column, in to a spreadsheet. The spreadsheet will determine the column precision for each sample.

4.1.11.3.2 If the instrument's MACRO is not used, a copy of the I:\ drive "Volatiles Determination Casefile Worksheet" (or equivalent) can be used. The casefile TAB can be used to calculate the column precision. The calibrator and control TABS are used to calculate the column precision for calibrator and control samples.

4.1.11.3.3 The values obtained from column 1 and column 2 must agree within 0.015g/100cc.

4.1.11.3.4 If the precision requirement is not met, the sample must be reanalyzed. If upon reanalysis, the column precision requirement is not met, instrument troubleshooting practices must be initiated and documented.

4.1.11.4 Quantitative Replicate Precision Criteria

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4.1.11.4.1 For case samples and when more than one replicate is performed for calibrator or control samples, calculate the mean value (columns 1 and 2) for each sample with a spreadsheet form.

4.1.11.4.2 The mean value for replicates must agree as described in the following table. If the precision requirement is not met, the sample must be reanalyzed

Results Range (g/100cc)	Precision (g/100cc)
0.02 - 0.10	0.010
0.11 - 0.20	0.015
0.21 - 0.30	0.020
0.31 - 0.50	0.030

4.1.11.4.3 For case samples, homogenization should be considered when a lack of replicate precision is observed.

4.1.11.4.4 If upon re-analysis, the replicate precision requirement for control sample(s) is not met, instrument troubleshooting must be initiated and documented.

4.1.11.5 High Ethanol Values

For samples above the highest calibrator used to establish calibration curve/table, the sample must be reanalyzed with a 0.5 dilution. The dilution factor is incorporated into final calculations.

4.1.11.6 Out of Range Control(s)

4.1.11.6.1 Single Value

4.1.11.6.1.1

When the value for a single control falls outside of either the required concentration or precision range and it is the only control that does so, the value may be considered an anomaly.

4.1.11.6.1.2

The analysis run may only be accepted if the anomaly is in excess of the required number of controls as described in section 4.1.7.3, *Per Analysis Run Control Requirements*.

4.1.11.6.2 Two or More Values

4.1.11.6.2.1

When more than one control value falls outside of the either the required concentration or precision range, the casework samples following the non-

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conforming controls must be reanalyzed.

4.1.11.6.2.2 The discipline leader should be consulted when more than one control falls outside of range to discuss appropriate options.

4.1.12 REPORTING OF RESULTS

4.1.12.1 General

4.1.12.1.1 Samples must be quantitated to a minimum of three decimal places (0.000).

4.1.12.1.2 The mean ethanol value is calculated as described in section 4.1.11.2.

4.1.12.1.3 The current uncertainty value must be subtracted from the mean ethanol value. This becomes the adjusted ethanol concentration.

4.1.12.1.4 Report values $<0.02\text{g}/100\text{cc}$ as "*none detected*".

4.1.12.2 Blood Ethanol Results

4.1.12.2.1 Report adjusted ethanol concentration, as grams of ethanol per 100cc of blood, truncated to two decimal places (0.00).

4.1.12.2.2 If the sample and/or sample vial does not comply with IDAPA 11.03.01, an appropriate comment must be noted on the analysis report. Examples of comments are described in section 4.1.12.6.1.

4.1.12.3 Urine Ethanol Results

4.1.12.3.1 The adjusted ethanol concentration must be multiplied by 0.67. Report mean value, as grams of ethanol per 67 mL of urine, truncated to two decimal places.

4.1.12.3.2 Report values $<0.02\text{g}/67\text{mL}$ as "*none detected*".

4.1.12.3.3 A qualifier statement "*Urine results may be of questionable value*" must be included in the analysis report.⁸

4.1.12.4 Vitreous Humor Ethanol

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4.1.12.4.1 Report adjusted ethanol concentration, as grams of ethanol per 100cc of vitreous humor, truncated to two decimal places (0.00).

4.1.12.4.2 No conversion to a blood alcohol value will be made.

4.1.12.5 Reporting of Qualitative Volatiles Results

The qualitative presence of other volatiles such as acetone, isopropyl alcohol, methyl alcohol, toluene and formaldehyde must be noted on the analysis report following the ethyl alcohol results.

4.1.12.6 Comments for Analysis Report

As appropriate and/or required, comments outlining actions, discrepancies and/or qualifiers must be included on the analysis report following the results of analysis. The following are examples of commonly used ones. Additional comments may be added as the need arises.

4.1.12.6.1 **Living Subjects or Unknown Status**

- *Collection tube(s) do not comply with IDAPA 11.03.01.*

This comment only applies when blood is collected since IDAPA does not address the collection tube for vitreous humor.

- *Sample does not comply with IDAPA 11.03.01.*

This comment must be used when the sample is clearly not blood.

4.1.12.6.2 **Deceased Subjects**

- *The lack of appropriate preservative in collection tube(s) makes this result questionable.*

- *The lack of appropriate preservative in collection tubes could adversely affect the reliability of this result.*

These comments can be applied to both blood and vitreous humor samples not collected in an IDAPA approved container. The IDAPA requirements only apply to evidentiary testing for living subjects; however, the high potential for ethanol production in non-preserved samples from deceased individuals makes a qualifier statement necessary.

4.1.12.6.3 **General Comments**

- *Collection kit forwarded for further analysis.*

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Specify where the kit will be forwarded.

▪ *Specimen unsuitable for testing.*

This comment can be used with questionable samples as described for living subjects in section 4.1.8.2.6.2 and for severely degraded postmortem samples.

4.1.13 ANALYSIS DOCUMENTATION

4.1.13.1 Volatiles Analysis Forms

4.1.13.1.1 Required QA spreadsheet form is located on the I:\ drive under International Management System\Toxicology\Toxicology Forms\... If desired, this form may be placed on the instrument's computer.

4.1.13.1.2 The case sample volatiles analysis spreadsheet must be included in case file with corresponding data.

4.1.13.1.3 The case sample volatiles analysis spreadsheet must contain the current analysis parameters.

4.1.13.1.4 The I:\ form includes spreadsheets for calibrator and control data. The spreadsheets for the calibrator and control data are centrally stored with original control and calibrator data.

4.1.13.1.5 The formatting for the volatiles analysis form must be such that ethanol values are reported to a minimum of three decimal places.

4.1.13.2 Quality Assurance Data

4.1.9.2.1 A copy of quality assurance data (calibrators and controls) need not be included in individual case files.

4.1.9.2.2 A packet containing data for response factor/calibration curve, controls and reference material will be prepared for each analysis run and stored centrally in the location designated for alcohol quality assurance data in the laboratory where the analysis was performed until archiving.

4.1.9.2.3 When necessary, a copy of the quality assurance data can be prepared from the centrally stored documents.

4.1.14 AUTHENTICATION OF REFERENCE MATERIALS

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- 4.1.14.1 Quantitative and Qualitative Volatile Reference Material
- 4.1.14.1.1 Reference material used for quantitative purposes must be traceable to NIST standards.
- 4.1.14.1.2 All available *Certificate of Analysis* for reference material will be stored centrally.
- 4.1.14.1.3 New lots of aqueous ethanol, aqueous mixed volatiles, and volatile reagent reference material must be included in duplicate in a minimum of one analysis run prior to official use.
- 4.1.14.1.4 The authentication data and data evaluation must be centrally stored.
- 4.1.14.1.5 **Aqueous Ethanol Solutions**
- 4.1.14.1.5.1 The *Certificate of Analysis*, together with a comparison of relative retention time and quantitation data, against existing calibrators, will serve as the qualitative and quantitative authentication of ethanol in the solution.
- 4.1.14.1.5.2 The new lot number can be accepted if the mean relative retention time for the new standard is ± 0.10 minutes and the mean concentration obtained falls within 6% of the target value (assayed) listed on the *Certificate of Analysis*.
- 4.1.14.1.5.3 Evaluation of data must be such that compliance with retention time and concentration requirements is apparent.
- 4.1.14.1.6 **Aqueous Mixed Volatile Reference Material**
- 4.1.14.1.6.1 The *Certificate of Analysis* for an aqueous mixed volatile reference solution along with a comparison to data from the previous runs will serve as the qualitative authentication of the components of the reference material mixture.
- 4.1.14.1.6.2 The solution prepared with a new lot number of volatile chemical reference material can be accepted if the mean

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relative retention time for each component in the new lot of reference material is ± 0.10 minutes. If ethanol is present in the mixture, the mean concentration must fall within 6% of the target value (assayed) listed on the *Certificate of Analysis*.

4.1.14.1.6.3 Evaluation of data must be such that compliance with retention time and concentration requirements is apparent.

4.1.14.1.7 **Volatile Reagent Reference Material**

4.1.14.1.7.1 For volatile reference material (acetone, ethanol, methanol, isopropanol, toluene...) used to prepare single constituent or mixed standard of volatiles, the qualitative authentication is established with the *Certificate of Analysis* and comparison of relative retention times.

4.1.14.1.7.2 The new lot number can be accepted if the mean relative retention time (RRT) for the new standard is ± 0.10 minutes from the RRT of existing qualitative standard components. The neat volatile reagents are diluted prior to analysis as described in section 4.1.5.3.2.

4.1.14.1.7.3 Evaluation of data must be such that compliance with retention time requirements is apparent.

4.1.14.1.7.4 If the volatile of interest is not listed in section 4.1.5.3.2, the volume added to a single component or multicomponent mixture must be optimized for the particular volatile. Documentation must be centrally stored.

4.1.14.2 **Blood Controls**

4.1.14.2.1 The Toxicology Program Discipline Leader or designee will characterize a new lot of blood controls.

4.1.14.2.1.1 The new lot will be analyzed in each ISP-FS laboratory involved in alcohol/volatiles analysis.

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- 4.1.14.2.1.2 Each ISP-FS laboratory will provide data from the analysis of a minimum of 20 samples. At least two bottles of control must be sampled.
- 4.1.14.2.1.3 The manufacturer's values will be acknowledged, however, the target value and range of a blood control lot will be established through a mean of all provided determinations.
- 4.1.14.2.1.4 The new blood lot number can be accepted if the mean relative retention time for the new control is ± 0.10 minutes of the RRT currently established for ethanol and the mean concentration obtained falls within the range provided in the manufacturer's package insert.
- 4.1.14.2.1.5 A 10% and 5% range will be calculated from the mean value of the determinations and used to evaluate accuracy on subsequent analysis. The 5% range will serve as a warning limit.
- 4.1.14.2.1.6 For blood controls that contain other volatiles (acetone, methanol, isopropanol, toluene) in addition to ethanol; the qualitative determination of the components must be established through the comparison of relative retention times from the previous run. The values must agree within ± 0.10 minutes.
- 4.1.14.2.1.7 Blood control *Package Inserts* will be stored centrally.

4.1.15 REFERENCES AND RECOMMENDED READING

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- 4.1.15.6 Perrine, D.M., *Depressants: Alcohol, Benzodiazepines, Barbiturates*. in: The Chemistry of Mind-Altering Drugs, pp. 113-129, ACS, Washington, DC, 1996.
- 4.1.15.7 Hobbs, W.R., Rall, T.W. and Verdoorn, T.A., *Drugs Acting on the Central Nervous System - Hypnotics and Sedatives; Ethanol*. in: Goodman and Gilman's The Pharmacological Basis of Therapeutics, pp. 361, 386-393, McGraw-Hill, 1996.
- 4.1.15.8 Idaho Administration Code, IDAPA 11.03.01, Rules Governing Alcohol Testing.
- 4.1.15.9 Jones, A.W., *Disposition and Fate of Ethanol in the Body*. in: Medical-Legal Aspects of Alcohol, edited by J.C. Garriott, pp. 87 - 90, Lawyers & Judges Publishing Co., Inc., 2003.
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- 4.1.15.12 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.

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- 4.1.15.14 Klaassen, C.D., *Nonmetallic Environmental Toxicants*. in: Goodman and Gilman's The Pharmacological Basis of Therapeutics, pp. 1681-1682, McGraw-Hill, 1996.
- 4.1.15.15 Klaassen, C.D., *Inhalants*. in: Principles of Forensic Toxicology, edited by Barry Levin, pp. 341-348, AACCC Press, 2003.
- 4.1.15.16 Baselt, R.C., *Ethanol*. in: Disposition of Toxic Drugs and Chemicals in Man, pp. 411-414, Biomedical Publications, 2004.
- 4.1.15.17 Dang, J.N., Statistical Analysis of Alcohol-related Driving Trends, 1982 - 2005, NHTSA Technical Report No. DOT HS 810 942, May 2008.

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Revision History

Section Four

Analysis of Alcohol and Common Volatile Solvents

4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles in Blood, Vitreous Humor and Urine by Dual Column Headspace Gas Chromatography

Revision #	Issue Date	Revisions
0	10/2001	
1	05-15-2002	Clarifications, coefficient of correlation change for system compatibility.
2	09-13-2002	Addition of analysis documentation section.
3	01-03-2003	Clarifications, refinement of analysis documentation section 4.1.10.
4	04-06-2004	Clarifications, acceptance criteria and quality assurance sections amended, authentication guidelines added.
5	06-16-2004	Addition to section 4.1.8.5.3. Modification of 4.1.8.11.4 (<i>duplicate</i> replaced with <i>replicate</i>)
6	12-29-2005	Modified format, updated and clarified quality assurance requirements.
7	05-07-2007	Updated QA measures, nomenclature and formatting.
8	08-20-2008	Updated for new instrumentation. Deviation in place prior to this date. Made running an internal standard blank following the high blood control an option instead of a requirement (4.1.7.3.2.2). Added uncertainty language. Clarified and consolidated sections.

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