

# Idaho State Police Forensic Services

## Approval for Quality System Controlled Documents



Discipline/Name of Document: Toxicology

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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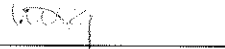
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## Section Four

### 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.<sup>6</sup> 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 drivers involved in fatal crashes that had a BAC of 0.08 or above decreased from 35% in 1982 to 20% in 1997 and leveled off thereafter.<sup>17</sup> 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.<sup>2,6,7</sup> Chronic excessive ingestion of ethanol is directly associated with serious neurologic and mental disorders such as brain damage, memory loss, sleep disturbances and psychoses.<sup>7</sup> 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.<sup>4</sup> The first mental processes to be affected are those that depend on training and previous experience.<sup>7</sup> 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.<sup>7</sup> 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.<sup>4</sup>

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.<sup>2,4,6</sup> Alcohol is soluble in both water and fat, a property that facilitates its diffusion through biological membranes.<sup>4</sup> 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.<sup>4,7</sup> 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.<sup>2,7</sup> 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.<sup>2</sup> 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.<sup>2</sup>

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.<sup>2</sup> 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.<sup>2</sup>

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.<sup>2</sup> In addition to gender, chronic abuse, prescription drugs and certain genetic factors can also influence the elimination rate.<sup>2,6,7</sup>

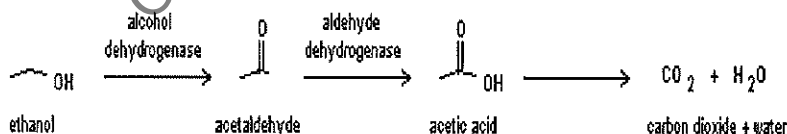


Figure 1. Metabolism of Ethanol.

Other commonly encountered toxic alcohols, alcohols other than ethanol that are not intended to be ingested, such as methanol and isopropanol, produce central nervous system (CNS) depressant effects but vary significantly in the degree.<sup>2,18</sup> Methanol (wood alcohol), commonly a component of model airplane fuel and windshield wiper fluid, causes relatively little intoxication compared to ethanol.<sup>2,6,18</sup> 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.<sup>2,6</sup> The formic acid leads to metabolic acidosis. Isopropanol (rubbing alcohol) is more toxic than ethanol with more prominent gastritis that includes pain, nausea, vomiting and hemorrhage.<sup>11,19</sup> Isopropanol is metabolized to acetone.<sup>2,4</sup> Note that elevated endogenous acetone may also be detected in the samples from a diabetic or fasting individual.

A variety of volatile chemicals may also be detected in samples from subjects that were inhaled either accidentally or intentionally.<sup>15</sup> For instance, toluene and acetone may be detected in subjects that come into contact with products such as aerosol paint and contact adhesives. 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.<sup>15</sup> Acetone is metabolized to acetate and formate. Inhaling of electronic cleaning products referred to as computer "dusters" can result in detectable levels of the aerosol propellants 1,1-Difluoroethane (HFC-152a) and 1,1,1,2-Tetrafluoroethane (HFC-134a).<sup>15,21</sup> The primary consequence of abuse is cardiovascular in nature.<sup>15</sup>

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 autosampler (HSA) for sample introduction. An aliquot of sample is placed into a headspace vial along with an aqueous 1-propanol internal standard in 1M Ammonium Sulfate. The sample vials are sealed and heated in a HSA. 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. The ammonium sulfate serves as a salting-out agent, thus improving the recovery of volatiles from the headspace.<sup>12,13,20</sup> An aliquot of the headspace vapor is injected into a GC with 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 calibration curve established by 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 and 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, difluoroethane and related compounds, via a headspace sampling gas chromatographic method.

### 4.1.3 EQUIPMENT

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

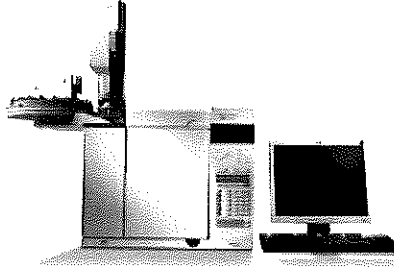


Figure 2. Gas Chromatograph

- 4.1.3.2 Agilent G1888 Headspace Sampler (Figure 3).

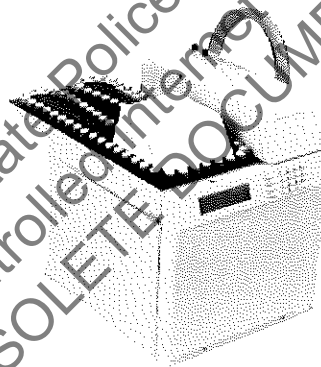


Figure 3. Headspace Analyzer

#### 4.1.3.3 Columns

- 4.1.3.3.1 Restek Rtx<sup>®</sup>-BAC1 (#18003: 30 meter X 0.32mm inner diameter (ID), 1.8 $\mu$ m film thickness (FT) or equivalent column)
- 4.1.3.3.2 Restek Rtx<sup>®</sup>-BAC2 (#18002: 30 meter X 0.32mm ID, 1.2  $\mu$ 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 $\mu$ L and 2000 $\mu$ L, respectively.

#### 4.1.4 REAGENTS

- 4.1.4.1 Distilled/Deionized water (free from volatiles of interest)
- 4.1.4.2 Ammonium Sulfate (Certified ACS Grade)

#### 4.1.5 REFERENCE MATERIAL

*When available, a certificate of analysis must be centrally stored.*

##### 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 sample. 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 Multicomponent Volatile Aqueous Solutions

Multicomponent solutions may be purchased or prepared as indicated below.

###### 4.1.5.2.1 **Commercially Obtained Multicomponent Solution**

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

4.1.5.2.1.2 When the multicomponent solution contains quantitative amounts of ethanol, it may simultaneously serve as an aqueous ethanol control.

###### 4.1.5.2.2 **Prepared Mixed Volatile Reference Solution**

Reference material used to prepare mixed volatile solution must be  $\geq 99\%$ . Refer to section 4.1.5.2.3 for the preparation of fluoroethane reference material.

4.1.5.2.2.1 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	≈100μL
Acetone	≈100μL
Ethanol	≈100μL
Ethyl Acetate	≈100μL
Methanol	≈500μL
Isopropanol	≈500μL
Toluene	≈50μL

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

4.1.5.2.2.2 Additional volatiles of interest may be used singularly or added to the mixed volatile solution.

4.1.5.2.2.3 The GC oven temperature conditions must provide for baseline separation for all components placed in the mixture.

#### 4.1.5.2.3 Fluoroethane Volatile Reference Solution

Fluoroethane reference solutions may be prepared from commercially obtained aerosol products. The product's MSDS must be obtained. Solutions may be used for as long as acceptable performance is obtained.

#### 4.1.5.3 Internal Standard Solution

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

4.1.5.3.1 Add approximately 800mL of DI water to a 1L volumetric flask. Add 132.14g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and mix to dissolve. Add 375μL 1-propanol. QS to 1L with distilled water.

4.1.5.3.2 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 MATRIX CONTROL MATERIAL

*The package insert must be centrally stored.*

**4.1.6.1 Ethanol or Multicomponent Whole Blood Control Material**

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

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

Level	Approximate Ethanol Range (g/100mL)
Low	0.03 – 0.10
High	0.13 – 0.40

4.1.6.2.3 A whole blood control containing ethanol with other volatiles can serve as a multicomponent control if the GC oven temperature program provides for baseline separation of all components.

**4.1.7 SAFETY CONCERNS**

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

**4.1.8 QUALITY ASSURANCE**

4.1.8.1 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 submitted in plastic bottles must be frozen for long-term storage.

4.1.8.2 The syringes on the Pipetter/Dilutor must be checked for accuracy and precision. Refer to Analytical Method 5.1 for pipette intermediate check and calibration requirements and options.

4.1.8.3 Refer to Analytical Method section 5.2 for balance intermediate check and calibration requirements.

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

4.1.8.4 Refer to manufacturer manuals for as-needed instrument maintenance procedures and troubleshooting measures.



- 4.1.8.5 Current source and lot number of controls and reference material must be documented.
- 4.1.8.6 Refer to Analytical Method 5.13 for quantitative ethanol reporting confidence interval/uncertainty.
- 4.1.8.7 Refer to Analytical Method 5.14 for authentication requirements for volatiles reference material and matrix controls.

#### 4.1.9 PRE-RUN COLLECTION KIT PROCESSING

##### 4.1.9.1 Collection Kit Description and Labeling

- 4.1.9.1.1 Note collection kit and container description on analysis coversheet form.
- 4.1.9.1.2 Note if it is apparent that the sample container does not appear to be the one originally included in collection kit.
- 4.1.9.1.3 Inspect inner seals on specimen container and note their condition on current coversheet form.
- 4.1.9.1.4 Note sample type (blood, urine, vitreous humor, other) on *Volatiles Analysis Coversheet*.
- 4.1.9.1.5 Note type and number of specimen container(s) (tube or bottle) on *Volatiles Analysis Coversheet*
- 4.1.9.1.6 Laboratory number must be placed on each sample container.
- 4.1.9.1.7 When more than one sample is present, label all samples present. Use "A", "B", etc. or comparable.

##### 4.1.9.2 Blood Specimen Collection Container Evaluation

- 4.1.9.2.1 Idaho Administrative Code, IDAPA 11.03.01 requires law enforcement agencies to have blood specimens, from living subjects, to be collected in a container containing *ten (10) milligrams of sodium fluoride per cubic centimeter of blood plus an appropriate anticoagulant.*<sup>8</sup>
- 4.1.9.2.2 The containers provided in ISP-FS kits comply with these requirements. It must, however, not be assumed that an ISP-FS kit contains specimen collection tubes it was supplied with.

4.1.9.2.3 Non-ISP-containers must be evaluated as to compliance. Information from the manufacturer of the container will indicate sodium fluoride concentration as well as the presence of an appropriate anticoagulant.

4.1.9.2.4 Note blood specimen container compliance on *Volatiles Analysis Coversheet*.

4.1.9.3 Blood Specimen Evaluation

4.1.9.3.1 IDAPA 11.03.01 requires blood to be reported as grams of alcohol per 100cc of whole blood. Although the absolute determination that the sample is whole blood is beyond the scope of this analytical method, when it is the analyst's opinion that the intended blood sample is serum or otherwise questionable, the analyst has the following options.

4.1.9.3.1.1

Option One

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

4.1.9.3.1.2

Option Two

The sample is analyzed for ethanol and other volatiles according to Analytical Method 4.2, Analysis of Solutions Containing Ethanol and Common Volatiles. The report, therefore, will make no mention of the sample having a biological origin.

**4.1.10 ANALYSIS PROCEDURE**

4.1.10.1 Calibration Curve/Table Requirements

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

4.1.10.1.2 The minimum low calibrator is to be in the nominal range of 0.02 to 0.05g/100mL.

4.1.10.1.3 The highest calibrator must be a 0.30g/100mL or greater.

- 4.1.10.1.4 Calibration table may be established in a separate sequence just prior to sequence containing case samples.
- 4.1.10.1.5 Ethanol calibrators should be analyzed in order of increasing concentration.
- 4.1.10.1.6 The least squares line resulting from the analysis of the ethanol calibrators must have a coefficient of correlation of  $\geq 0.998$ .
- 4.1.10.1.7 Each ethanol calibrator may have more than one replicate.
- 4.1.10.1.7.1 In the sequence table, on the **Update RF** column, select "replace" for each of the first set of calibrators. If a second set of calibrators is run, the results are to be "averaged".
- 4.1.10.1.8 If data from a calibrator is not usable, the remaining data can be used to establish the response factor provided that requirements in 4.1.10.1.1, 4.1.10.1.2, 4.1.10.1.3 and 4.1.10.1.6 have been met.
- 4.1.10.1.9 A calibration curve/table is valid for 14 days, provided:
- ✓ Values for required controls fall within acceptable ranges.
  - ✓ The same preparation of internal standard solution used for the calibration run is used.
- 4.1.10.1.10 Once established, analysts not involved in establishing the calibration curve/table may use the established calibration table.
- 4.1.10.1.11 An analysis run may include case samples prepared by more than one analyst.
- 4.1.10.1.12 The internal standard blank should follow the highest ethanol calibrator.
- 4.1.10.2 Analysis Run Control and Blank Requirements
- 4.1.10.2.1 **Initial Run with Calibration Curve**  
For a run with a newly established calibration curve, a low (defined in 4.1.6.2.2) ethanol containing blood control must be included with the first ten samples

(20 vials) before proceeding on with the run. The low control must be run in duplicate.

4.1.10.2.2 **Additional Runs with Existing Calibration Curve**  
For analysis runs utilizing an existing calibration curve, a low and high ethanol-containing blood control, in duplicate, must be included with the first 10 samples before proceeding with additional samples.

4.1.10.2.3 **All Analysis Runs**

4.1.10.2.3.1 Each analysis run must contain an internal standard blank.

4.1.10.2.3.2 For analysis run consisting of more than 10 case samples (20 vials), a minimum of one blood or aqueous ethanol-containing control must be run with each additional 10 samples.

4.1.10.2.3.3 Each analysis run must include either an *aqueous* or *blood* multicomponent volatile mix.

4.1.10.2.3.4 A commercially obtained **quantitative multicomponent** volatile mix may serve as both an *aqueous ethanol control* and a *multicomponent mixture*.

4.1.10.3 **Sample Preparation**

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

4.1.10.3.2 Sample preparation must take place in a laminar flow hood or biological safety cabinet.

4.1.10.3.3 Place blood sample container on rocker for a minimum of two minutes.

4.1.10.3.4 If a blood sample appears to be coagulated, the sample may require homogenization in a tissue grinder, or equivalent.

4.1.10.3.5 Prepare pipetter/dilutor for use.

4.1.10.3.6 All case samples must be analyzed in duplicate. Calibrators may be run in duplicate. Refer to section 4.1.10.2 for Positive Control replicate requirements.

4.1.10.3.7 Use Pipetter/Dilutor to dispense 250 $\mu$ L of case sample, positive control, or calibrator solution, along with 2000 $\mu$ L of internal standard (ISTD), into labeled headspace vial and apply seal.

4.1.10.3.8 For internal standard blank, dispense 250 $\mu$ L of DI water along with 2000 $\mu$ L of internal standard (ISTD) into labeled headspace vial and apply seal.

#### 4.1.10.4 Instrument Run Preparation

4.1.10.4.1 Open **Sequence Table**. It is recommended that each analyst create, not share, a Sequence. This reduces the possibility of the Sequence being modified without their knowledge.

4.1.10.4.2 Into Sequence log table, enter the sample case numbers, ethanol calibrators, volatiles single constituent reference material, volatile reference material mixtures, blank(s) and controls.

4.1.10.4.3 Load samples, calibrators, blank(s), reference material(s) and controls onto the headspace sampler carousel rack as noted in the sequence table.

4.1.10.4.4 The sequence information should be verified prior to starting the instrument.

#### 4.1.10.5 Instrument Parameters

4.1.10.5.1 Refer to current instrument method for gas chromatograph and headspace analyzer analysis parameters.

4.1.10.5.2 Analysis method must be stored centrally (hardcopy and/or electronically) each time the method is updated.

### 4.1.11 ACCEPTANCE CRITERIA

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

- 4.1.11.2 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.
- 4.1.11.3 When a control value falls outside of either the required concentration or precision range, the casework samples following the non-conforming control(s) must be reanalyzed.
- 4.1.11.4 Qualitative Accuracy Criteria  
The qualitative presence of ethanol, or other volatile substances, can be established if the retention time for a specimen is within  $\pm 0.10$  minutes of the retention time of the reference compound in question. This criterion should be designated in the instrument's data station analysis method.
- 4.1.11.5 Quantitative Accuracy Criteria  
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 for blood controls are determined as described in Analytical Method 5.14.
- 4.1.11.6 Column Precision Criteria
- 4.1.11.6.1 The ethanol values obtained from column 1 and 2 must agree within 0.0150g/100cc.
- 4.1.11.6.2 If the precision requirement is not met, the sample must be reanalyzed. If upon reanalysis, the column precision requirement is not met, the source of the problem will be pursued. One possible cause is a system leak.
- 4.1.11.7 Quantitative Replicate Precision Criteria
- 4.1.11.7.1 The mean value for replicate analysis 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)
0.0200 - 0.1099	0.0100
0.1100 - 0.2099	0.0150
0.2100 - 0.3099	0.0200
0.3100 - 0.5000	0.0300

- 4.1.11.7.2 If upon re-analysis, the replicate precision requirement for control sample(s) is not met, troubleshooting must be initiated and documented. Case samples may require additional homogenization.

4.1.11.7.3 If desired, the BAC CALCULATION WORKSHEET.xls can be installed on the instrument's computer. A MACRO is available which can enter the duplicate ethanol concentrations for each sample and column.

#### 4.1.12 REPORTING OF RESULTS

##### 4.1.12.1 General

The three decimal place truncated mean ethanol value, as determined by this method, will be reported along with the  $\pm$  95% confidence interval range in the form of Coefficient of Variation (CV%). The determination of the confidence interval is addressed in Analytical Method 5.13.

##### 4.1.12.2 Blood Ethanol Results

4.1.12.2.1 Report mean ethanol concentration, as grams of ethanol per 100cc of blood, truncated to three decimal places  $\pm$  CV%.

4.1.12.2.2 Report values  $<0.020\text{g}/100\text{cc}$  as "*none detected*".

4.1.12.2.3 If the sample and/or sample vial clearly does not comply with IDAPA 11.03.01, an appropriate comment must be noted on the analysis report.

##### 4.1.12.3 Urine Ethanol Results

4.1.12.3.1 The four decimal place mean ethanol value must first be multiplied by 0.67.

4.1.12.3.2 Report mean ethanol value as grams of ethanol per 67mL of urine truncated to three decimal places  $\pm$  CV%.

4.1.12.3.3 Report values  $<0.020\text{g}/67\text{mL}$  as "*none detected*".

4.1.12.3.4 A qualifier statement "*Urine results may be of questionable value*" **must** be included in the analysis report.<sup>8</sup>

##### 4.1.12.4 Vitreous Humor Ethanol

4.1.12.4.1 Report mean ethanol concentration, as grams of ethanol per 100cc of vitreous humor, truncated to three decimal places (0.000)  $\pm$  CV%.

4.1.12.4.2 Report values <0.020g/100cc as "*none detected*".

4.1.12.4.3 No conversion to a blood alcohol value will be made on the report.

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 will 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 can be included on the analysis report following the results of analysis.

**4.1.13 ANALYSIS DOCUMENTATION**

4.1.13.1 Volatiles Analysis Forms

4.1.13.1.1 Required spreadsheet form for calibrator, controls and case sample can be located under I:\International Management System\Toxicology\Toxicology Forms

4.1.13.2 Quality Assurance Data

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

4.1.13.2.2 A packet containing spreadsheets and 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.

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

**4.1.14 REFERENCES AND RECOMMENDED READING**

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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	Initial version.
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.
9	09-07-2009	Clarified uncertainty and QA wording. Updated background, scope and references. Authentication section moved to AM 5.14.