

**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 in Blood, Vitreous Humor and Urine by Dual Column Headspace Gas Chromatography

Revision #	Issue Date	History
0	10/01	
1	05-15-02	Clarifications, coefficient of correlation change for system compatibility.
2	09-13-02	Addition of analysis documentation section.
3	01-03-03	Clarifications, refinement of analysis documentation section 4.1.10.
4	04-06-04	Clarifications, acceptance criteria and quality assurance sections amended, authentication guidelines added.

Approval

Technical Leader:

Susan C. Williamson
Susan C. Williamson

Date:

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Issuance

QC Manager:

Rick D. Groff
Rick D. Groff

Date:

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Idaho State Police
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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

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), a problem that plagues every country. 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 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 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 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

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 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}

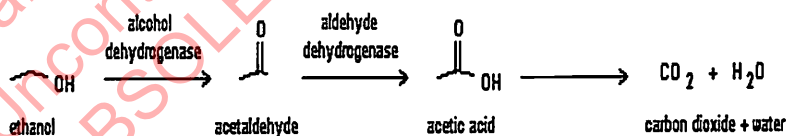


Figure 1. Metabolism of Ethanol.

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 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 blood, vitreous humor and urine is accomplished with a gas chromatograph (GC) which uses a headspace analyzer for sample introduction. Samples, controls and standards are sealed into vials that contain an aqueous n-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 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 n-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 n-propanol internal standard.

4.1.2 SCOPE

This method describes the Idaho State Police Forensic Services (ISP-FS) standard operating procedure for the analysis of aqueous samples (blood, vitreous humor and urine) for the presence of volatile compounds including methanol, ethanol, acetaldehyde, acetone, isopropanol, toluene and related compounds, via a headspace sampling gas chromatographic method. IDAPA 11.03.01 requires that determinations for ethyl alcohol for legal purposes be performed on blood samples.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.

4.1.3 EQUIPMENT

4.1.3.1 Perkin Elmer Auto System XL Gas Chromatograph (GC)

4.1.3.2 Columns

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 FT or equivalent column)

- 4.1.3.3 Perkin Elmer HS-40 or HS-110 Headspace Autosampler (figures 2 and 3)
- 4.1.3.4 PE Workstation Software, TotalChrom Version 6.2.0 or more recent version/upgrade.
- 4.1.3.5 Headspace (HS) vials (P-E B0104236 or equivalent)
- 4.1.3.6 Safety Closures for HS vials {PTBE coated rubber septa, crimp caps and star springs} (P-E B0104240 or equivalent)



Figure 2. HS-40



Figure 3. HS-110

- 4.1.3.7 Hand Crimper or Bench Top Crimper
- 4.1.3.8 Hamilton MICROLAB 503A or equivalent semi-automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250 μ L and 2000 μ L, respectively.

4.1.4 CONTROLS AND CALIBRATION STANDARDS

4.1.4.1 Whole Blood Control (\cong g/100cc)

Whole Blood Volatiles Control (BIO-RAD 565 or comparable)

BIO-RAD kit includes a Level 1 containing \cong 0.08 ethanol and a Level 2 containing \cong 0.15 ethanol for use as a quantitative control. Level 2 also contains acetone, methanol and isopropanol standards which this method utilizes qualitatively.

4.1.4.2 Aqueous Ethanol Calibration Standards (\cong g/100cc)

Options: 0.01, 0.025, 0.05, 0.08, 0.10, 0.20, 0.30, and 0.40 (Cerilliant or comparable). (As required by 4.1.8.10.1)

4.1.4.3 Aqueous Control (\cong g/100cc)

Multicomponent Alcohol Calibration Kit (Cerilliant #A-054 or comparable). Cerilliant kit includes an aqueous 0.05, 0.10 or 0.40 ethanol for use as a quantitative control and acetone, methanol and isopropanol standards which this method utilizes qualitatively.



4.1.5 REAGENTS

A certificate of analysis should be requested for all chemicals.

- 4.1.5.1 1-Propanol (Acros/Fisher Scientific # 23207-0010, #A996-1 or equivalent)
- 4.1.5.2 Acetone (Fisher #A18-500 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 Ethanol, 200 proof (Sigma 45,984-4)
- 4.1.5.6 Methanol (Fisher #A412-500 or equivalent)
- 4.1.5.7 Toluene (Fisher T324-500 or equivalent)
- 4.1.5.8 Ammonium Sulfate (Fisher #A702-500 or equivalent)
- 4.1.5.9 Sodium Fluoride (Fisher #S299-500 or equivalent)



4.1.6 SAFETY CONCERNS

- 4.1.6.1 Blood samples should be processed and chemicals handled 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 (NH₄)₂SO₄
Dissolve 132.14g (NH₄)₂SO₄ in approximately 800mL of to a 1L volumetric flask.
Add 1g sodium fluoride {optional}.
Add 375μL 1-propanol.
QS to 1L with distilled water.
Solution is stable for 2 months.

- 4.1.7.2 Qualitative Volatile Standard Mix Solution
Add approximately 200mL of DI water to a 250mL volumetric flask.
Add one or more of the following volatiles, *as needed*:
100 μL acetaldehyde
100 μL acetone
500 μL methanol
500 μL isopropanol
500 μL ethanol
50μL toluene
QS to 250mL.
Solution is stable for 1 year

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 Pipetter/Dilutor Set-up

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

4.1.8.3 Preparation of Calibration Standards

- 4.1.8.3.1 Label HS vials for with each ethanol calibration standard. A minimum of three points of calibration should be established.
- 4.1.8.3.2 Use Pipetter/Dilutor to dispense 250 μ L of aqueous ethanol standard and 2000 μ L of internal standard (ISTD) into each labeled headspace vial.
- 4.1.8.3.3 Seal **immediately** with crimp cap as illustrated in figure 4 (or equivalent).

4.1.8.4 Preparation of Quality Assurance Samples

4.1.8.4.1 Water Blank

- 4.1.8.4.1.1 Label HS vial with *water blank*.
- 4.1.8.4.1.2 Add 2000 μ L DI water to labeled headspace vial.
- 4.1.8.4.1.3 Seal **immediately** with crimp cap as illustrated in figure 4 (or equivalent).

4.1.8.4.2 Internal Standard Blank

- 4.1.8.4.2.1 Label HS vial with *ISTD blank*.
- 4.1.8.4.2.2 Use Pipetter/Dilutor to dispense 2000 μ L of internal standard (ISTD) into labeled headspace vial.
- 4.1.8.4.2.3 Seal **immediately** with crimp cap as illustrated in figure 4 (or equivalent).

4.1.8.4.3 Blood and Aqueous Controls

- 4.1.8.4.3.1 Label HS vials for appropriate whole *blood and aqueous controls*.

- 4.1.8.4.3.2 Use Pipetter/Dilutor to dispense 250 μ L of blood control and 2000 μ L 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 (or equivalent).

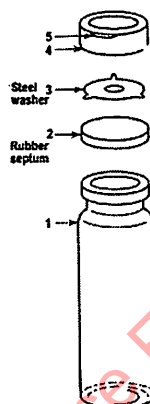


Figure 4. Crimp cap assembly

4.1.8.5 Initial Processing of Samples

- 4.1.8.5.1 Note sample container description on analysis coversheet.
- 4.1.8.5.2 Open the sample collection kit and remove the inner compartment.
- 4.1.8.5.3 Inspect inner seals and note their condition on the analysis coversheet.
- 4.1.8.5.4 Place laboratory number on each sample container.
- 4.1.8.5.5 When two samples are present, the samples should be labeled "A" and "B" or equivalent. Note container type(s) on analysis coversheet.
- 4.1.8.5.6 When the sample is blood, the sample vial and contents should be examined to determine compliance with IDAPA 11.03.01.⁸ Note compliance on analysis coversheet.
- 4.1.8.5.6.1 When the opinion of the analyst is the sample is serum or otherwise questionable, the analyst has the two options. Option one is to not analyze the sample and place the comment "sample unsuitable for analysis" on the analysis report. Option two is analyze the sample and place a qualifier on the analysis report to advise that "the sample does not comply with IDAPA 11.03.01".
- 4.1.8.5.6.2 Samples, which clearly do not have the appearance of blood, can be analyzed for

ethanol and other volatiles utilizing SOP 4.2.

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

4.1.8.6 Preparation for Analysis

4.1.8.6.1 Label two HS vials with the laboratory number.

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

4.1.8.7 Addition of Sample to Headspace Vials.

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

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

4.1.8.8 Preparation for Run

4.1.8.8.1 **Open Sequence Editor.**

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

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

4.1.8.9 Analysis Parameters

4.1.8.9.1 Refer to instrument METHOD printouts for gas chromatograph and headspace analyzer analysis parameters.

4.1.8.9.2 Analysis method printouts must be stored centrally.

4.2.8.10 Calibration

4.2.8.10.1 A minimum of three ethanol calibrators must be included in each run. The calibrators chosen should characterize the entire range of interest.

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

4.1.8.11 Acceptance Criteria

4.1.8.11.1 **Acceptance of Analysis Run**

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

4.1.8.11.1.2 If calibration standards are run in duplicate, it is not required that duplicate calibration points are included as long as linearity requirement is met.

4.1.8.11.2 **Qualitative Accuracy Criteria**

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

4.1.8.11.3 **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 are determined as described in section 4.1.11.

4.1.8.11.4 **Quantitative Precision Criteria**

The results obtained from duplicate analysis must agree within 0.01g/100cc for values up to 0.200g/100cc. For values above 0.200g/100cc, the precision criterion is 0.015g/100cc. If the precision requirement is not met, the sample is reanalyzed. Homogenization should be a considered.

4.1.8.11.5 **High Ethanol Values**

When an elevated ethanol value is obtained, appropriate calibrators must bracket the value. For instance, if a 0.38g/100cc blood ethanol value is determined, a 0.40g/100cc calibrator must be included in the run. The analyst has the option to either re-analyze the sample with a 0.40 calibrator or dilute the sample by 0.5. All samples above 0.40/100cc should be reanalyzed with a 0.5 dilution. The dilution factor should be incorporated into final calculations.

4.1.8.12 Reporting of Quantitative Ethanol Results

4.1.8.12.1 **Blood**

Samples are quantitated to a minimum of three significant figures. Report truncated mean value, of grams of ethanol per 100cc of blood, to two significant figures. Report values $< 0.02\text{g}/100\text{cc}$ as **none detected**. If the sample and/or sample vial does not comply with IDAPA 11.03.01, this should be noted on the analysis report.

4.1.8.12.2 Urine

Samples are quantitated to three significant figures. Result obtained should be multiplied by 0.67. Report truncated mean value, as grams of ethanol per 67 mL of urine, to two significant figures. Report values <0.02g/67mL as "*none detected*". A qualifier statement such as "*Urine results may be of questionable value*" must be included in the report.⁸

4.1.8.12.3 Vitreous Humor

Samples are quantitated to a minimum of three significant figures. Report truncated mean value, as grams of ethanol per 100mL of vitreous humor, to two significant figures. No conversion to a blood alcohol value should be made. Report values <0.02g/100cc as "*none detected*".

4.1.8.13 Reporting of Qualitative Volatiles Results

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

4.1.8.14 Comments for Analysis Report

As appropriate and/or required, comments outlining actions, discrepancies and/or qualifiers should be included on the analysis report following the results of analysis.

Examples of comments include:

- *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 should be used when the sample is clearly not blood.
- *Collection kit forwarded for further analysis.*
Specify where the kit will be forwarded.
- *Specimen unsuitable for testing.*

4.1.9 QUALITY ASSURANCE**4.1.9.1 General**

4.1.9.1.1 Blood or vitreous samples are to be stored under refrigeration while at the laboratory. Urine samples can be either refrigerated or frozen. Urine samples should be frozen for long-term storage.

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

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

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

4.1.9.2 Per Analysis Run Control Requirements

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

4.1.9.2.2 A blood control must be run in duplicate with the first 10 samples (20 vials).

4.1.9.2.3 A minimum of one blood control must be run with each additional 10 samples (20 vials).

4.1.9.2.4 A blood control containing ethanol with or without other volatiles substances meets the "per run" requirement.

4.1.9.3 Periodic Control Requirements

4.1.9.3.1 The blood control concentration(s) should be varied periodically.

4.1.9.3.2 An aqueous control sample may periodically be substituted for a blood control provided the requirement of two blood controls per batch is met. A commercial multi-component mixture containing ethanol meets this requirement.

4.1.9.3.3 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. Gas Chromatograph temperature program must provide for baseline separation of volatile compounds of interest as indicated by analysis of multicomponent mixtures.

4.1.9.4 Monitoring of Control Values

4.1.9.4.1 On a monthly basis, calculate the mean and standard deviation of control samples. The data serve as a continual check of manufacturer-supplied values.

4.1.9.4.2 All control data will be provided monthly to the Discipline Leader for the Toxicology Program.

4.1.10 ANALYSIS DOCUMENTATION

4.1.10.1 A packet containing original data for controls and standards will be prepared for each analysis run and stored centrally in the file designated for alcohol quality assurance data in the laboratory where the analysis was performed until archiving.

- 4.1.10.2 A copy of controls and standards need not be included in individual case files. When necessary, a copy of the control and standard printouts can be prepared from the centrally stored document.

4.1.11 AUTHENTICATION OF REFERENCE MATERIALS

4.1.11.1 Quantitative and Qualitative Volatile Standards

- 4.1.11.1.1 Standards for quantitative purposes must be traceable to NIST standards (or comparable).

- 4.1.11.1.2 *Certificate of Analysis* for all standards will be stored centrally.

- 4.1.11.1.3 New lots of aqueous ethanol, aqueous mixed volatiles, and volatile reagent standards should be included in duplicate in a minimum of one analysis run prior to official use.

- 4.1.11.1.4 Standards authenticated prior to the start date of this SOP revision can be used until consumed. The authentication data must be centrally stored.

4.1.11.1.5 **Aqueous Ethanol Standards**

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 standard. 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 listed on the *Certificate of Analysis*.

4.1.11.1.6 **Aqueous Mixed Volatile Standards**

The *Certificate of Analysis* for an aqueous mixed volatile standard along with a comparison to data from the previous runs will serve as the qualitative authentication of the components of the standard. The solution prepared with a new lot number of volatile chemical standard can be accepted if the mean relative retention time for the new standard is ± 0.10 minutes. If ethanol is present in the mixture, the mean concentration must fall within 6% of the target value listed on the *Certificate of Analysis*.

4.1.11.1.7 **Volatile Reagent Standards**

For reagent standards (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. 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 the qualitative standard components. The reagent should be diluted prior to analysis as described in section 4.1.7.2.

4.1.11.2 Blood Controls

4.1.11.2.1 The Toxicology Program Discipline Leader or designee will characterize a new lot prior to release to individual analysts.

4.1.11.2.1.1 The target value and range of a blood control lot will be established through the analysis with a minimum of 20 determinations.

4.1.11.2.1.2 The new blood lot number can be accepted if the mean ($n \geq 20$) relative retention time for the new control is ± 0.10 minutes and the mean ($n \geq 20$) concentration obtained falls within the range provided in the manufacturer's package insert.

4.1.11.2.1.3 A 10% range will be calculated from the laboratory determined mean value and used to evaluate accuracy on subsequent analysis. 5% should serve as a warning limit.

4.1.11.2.1.4 For blood controls that contain other volatiles (acetone, methanol, isopropanol, toluene) in addition to ethanol; the qualitative determination of the components should be established through the comparison of relative retention times from the previous run. The values should agree within ± 0.10 minutes.

4.1.11.2.2 Subsequent to analysis by the Discipline Leader, new blood control lots must be analyzed by the analyst/laboratory utilizing the new blood control a minimum of once in duplicate to verify values are in agreement with acceptance criteria.

4.1.11.2.2.1 The data must be provided to the Discipline Leader prior to official use of a new lot of blood control.

4.1.11.2.3 *Package Inserts* will be stored centrally.

4.1.11.2.4 Controls authenticated prior to the start date of this SOP revision can be used until consumed. The authentication data must be centrally stored.

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1	05-15-02	Clarifications, coefficient of correlation change for system compatibility.
2	09-13-02	Addition of analysis documentation section.
3	01-03-03	Clarifications, refinement of analysis documentation section 4.1.10.
4	04-06-04	Clarifications, acceptance criteria and quality assurance sections amended, authentication guidelines added.

Approval

Technical Leader: _____ **Date:** _____
 Susan C. Williamson

Issuance

QC Manager: _____ **Date:** _____
 Rick D. Groff