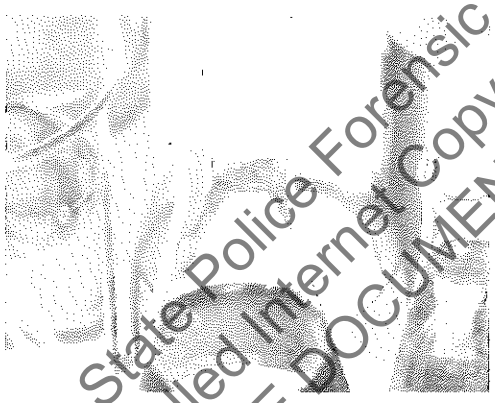
Idaho State Police
Forensic Services

Approval for Quality System Controlled Documents



Discipline/Name of Document: Toxicology
4.2 Analysis of Solutions containing Ethanol and Common Volatiles

Revision Number: 3

Issue Date: 5/07/2007

APPROVED BY: Corinna C Ousley
Quality Manager

5/7/07
Date Signed

Section Four
Analysis of Alcohols and Common Volatile Solvents

4.2 Analysis of Solutions Containing Ethanol and Common Volatiles

4.2.1 BACKGROUND

The need to establish the ethyl alcohol concentration and/or the presence of other commonly encountered volatiles in a beverage or solution may arise from ABC violations (Idaho Code 23-611, 23-1002, 23-1303, ...), under-age consumption (Idaho Code 23-603, 23-604), open-container violations (Idaho Code 23-505, 23-1333), poisonings and/or an endless variety of situations including questionable samples submitted as blood or other physiological fluid. In addition, ethyl alcohol concentration must be verified in simulator solutions used for breath testing instruments (IDAPA 11.03.01).

4.2.2 SCOPE

This method describes the analysis of alcoholic beverages and solutions said to contain a specified amount of ethyl alcohol and of unknown solutions via a headspace sampling gas chromatographic method. Unknown solutions may also contain other volatiles such as acetone, methanol, isopropanol and toluene, which can be qualitatively identified with this method. 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 a gas chromatograph (GC) in a dual column configuration. The GC serves to separate out the components of the solution as a function of their chemical properties. The separated components are identified qualitatively on the basis of the retention time determined for each of the columns. Quantitation of ethanol is accomplished through area percent data obtained from a flame ionization detector (FID). The quantitative result is based on a minimum of a three-point calibration curve, which uses the peak area ratio between ethanol and the n-propanol internal standard. These solution samples can be included as part of a toxicology alcohol determination run utilizing SOP 4.1 provided that quality assurance requirements are met. In addition, if this method is applied specifically for the qualitative identification of volatiles other than ethanol, ethanol calibrators and controls need not be run.

4.2.3 EQUIPMENT

4.2.3.1 Perkin Elmer Auto System XL Gas Chromatograph (GC)

4.2.3.2 Columns

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

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

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

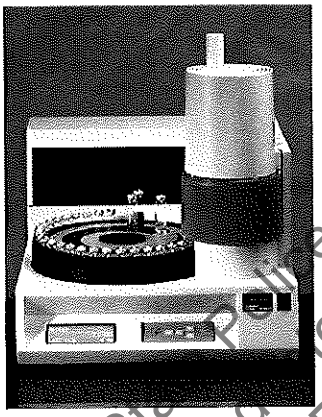


Figure 2. HS-40

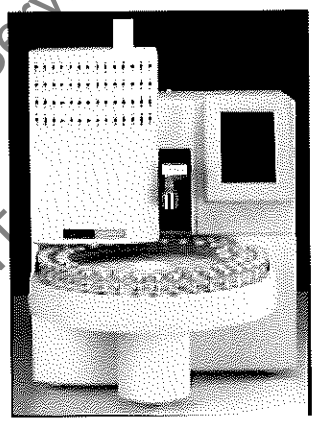


Figure 3. HS-110

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

4.2.3.5 Hand Crimper

4.2.3.6 Semi-automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250µL and 2000µL, respectively.

4.2.3.7 GC-Headspace vials

4.2.3.8 Safety Closures {PTBE septa, crimp caps and star springs}

4.2.4 SAFETY CONCERNS

4.2.4.1 Samples must be processed according to safety guidelines in the *Chemical Hygiene and Safety Manual.*

4.2.5 REAGENTS

4.2.5.1 1-Propanol (≥99%)

4.2.5.2 Acetone (≥99%)

4.2.5.3 Acetaldehyde (≥99%)

4.2.5.4 Isopropanol (2-Propanol) (≥99%)

4.2.5.5 Methanol (≥99%)

4.2.5.6 Toluene (≥99%)

4.2.5.7 Ammonium Sulfate (Certified ACS Grade)

4.2.6 REFERENCE MATERIAL

Record the preparation of all solutions on reagent log.

4.2.6.1 Ethanol Calibration Standards

Aqueous Ethanol Standards (g/100mL)

As available, 1-mL ampules containing solutions at concentrations of ≅0.025, 0.05, 0.08, 0.10, 0.20, 0.30, and 0.40 (Cerilliant or equivalent)

4.2.6.2 Ethanol Control Standard

Aqueous Ethanol Standards (g/100mL)

As available, 1-mL ampules containing concentrations of 0.02 to 0.40. If available, vendor/source and/or lot number not used to prepare calibration standards must be obtained.

4.2.6.3 Aqueous Multicomponent Mixture (≅g/100cc)

Multicomponent mixture can be obtained commercially and/or prepared from reagents as described below.

4.2.6.3.1 **Multicomponent Kit**

(Cerilliant #A-054 or comparable).

Cerilliant kit includes an aqueous 0.05, 0.10 or 0.40 ethanol for use as a quantitative ethanol control and acetone, methanol and isopropanol standards which this method utilizes qualitatively.

4.2.6.3.2 **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:

100µL acetaldehyde
100µL acetone
500µL methanol
500µL isopropanol
500 µL ethanol
100µL toluene.

QS to 250mL.

Solution is stable for 1 year.

As the need arises, other volatiles can be included in this mixture or in a single constituent solution. The

volatile is analyzed to determine chromatography characteristics including the retention time. Retention time must be programmed into analysis methods.

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

4.2.6.4.1 Add approximately 800mL of DI water to a 1L volumetric flask. Add 132.14g $(NH_4)_2SO_4$ and mix to dissolve. Add 375µL 1-propanol. QS to 1L with distilled water.

4.2.6.4.2 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.

4.2.7 ANALYSIS PROCEDURE

4.2.7.1 General

4.2.7.1.1 Solutions covered under this SOP can be included as part of a routine toxicology alcohol analysis run.

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

4.2.7.1.3 If analysis is for volatiles other than ethanol, ethanol calibrators need not be included in the analysis run.

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

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

4.2.7.2 Pipetter/Dilutor Set-up

4.2.7.2.1 Switch on power.

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

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

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

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

4.2.7.3 Preparation of Blanks and Mixed Standard

4.2.7.3.1 Water Blank

4.2.7.3.1.1 Label test vial with *water blank.*
4.2.7.3.1.2 Add 2mL DI water to labeled headspace vial.
4.2.7.3.1.3 Seal **immediately** with crimp cap.

4.2.7.3.2 Internal Standard Blank
4.2.7.3.2.1 Label test vial with *ISTD blank.*
4.2.7.3.2.2 Use Pipetter/Dilutor to dispense 2000µL of internal standard (ISTD) into labeled headspace vial.
4.2.7.3.2.3 Seal **immediately** with crimp cap.

4.2.7.3.3 Aqueous Ethanol Controls
4.2.7.3.3.1 Label appropriate number of headspace vials for *aqueous ethanol controls (1, 2,...).*
4.2.7.3.3.2 Use Pipetter/Dilutor to dispense 250µL of aqueous control and 2000µL of internal standard (ISTD) into each labeled headspace vial.
4.2.7.3.3.3 Seal **immediately** with crimp cap.

4.2.7.3.4 Mixed/Other Volatiles Standard Solution
4.2.7.3.4.1 Label headspace vial with qualitative volatiles solution.
4.2.7.3.4.2 Use Pipetter/Dilutor to dispense 250µL of mixed volatile solution and 2000µL of internal standard (ISTD) into labeled headspace vial.
4.2.7.3.4.3 Seal **immediately** with crimp cap.

4.2.7.4 Ethanol Calibration Standard Preparation
4.2.7.4.1 Label vials for standards.
4.2.7.4.2 Use Pipetter/Dilutor to dispense 250µL of appropriate ethanol concentration and 2000µL of internal standard (ISTD) into each labeled headspace vial.
4.2.7.4.3 Seal **immediately** with crimp cap.
4.2.7.4.4 Establish ethanol calibration plot with a minimum of three calibration points.

4.2.7.5 Initial Processing of Specimens
4.2.7.5.1 Inspect and document the condition of seals.
4.2.7.5.2 Remove sample(s) container from packaging and place laboratory number on each sample.

4.2.7.6 Preparation of Samples for Analysis

4.2.7.6.1 Label two headspace vials with the laboratory number.

4.2.7.6.2 Dilute alcoholic beverages and unknown solutions as necessary. The sample must be diluted for the value to fall on calibration curve. Generally, beer and wine should be diluted 50:1 with DI water and distilled beverages (≥ 16% w/v or 20% v/v) diluted 100:1. If available, the dilution of unknown solutions should be based on sample history.

4.2.7.6.3 Breath testing simulator solutions and samples, which appear to be serum, do not require dilution.

4.2.7.7 Addition of sample to headspace vials.

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

4.2.7.7.2 Seal headspace vials **immediately** with crimp caps.

4.2.7.8 Preparation for Run

4.2.7.8.1 Open **Sequence Editor**

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

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

4.2.7.9 Instrument Acquisition Parameters

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

4.2.7.9.2 Parameters are at the discretion of the analyst and must be optimized for the instrument.

4.2.7.9.3 GC oven parameters must provide for the baseline separation of commonly encountered volatiles in the test mixtures described in sections 4.2.6.3.

4.2.7.9.4 Each laboratory must maintain a centrally stored current METHOD printout.

4.2.7.10 Calibration

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

4.2.7.10.2 Ethanol calibrators must be analyzed in order of increasing concentration.

4.2.7.11 Acceptance Criteria

4.2.7.11.1 **Acceptance of Analysis Run**

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

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

4.1.7.11.2 **Qualitative Accuracy Criteria**

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

4.1.7.11.3 **Quantitative Accuracy Criteria**

The quantitative ethanol results for a batch of samples can be accepted if the values obtained for aqueous control samples fall within ±10% of target value on Certificate of Analysis.

4.2.7.11.4 **Quantitative Precision Criteria**

The results obtained from duplicate analysis must agree within 0.010g/100mL. For breath testing solutions, the results between different bottles of solution must also agree within 0.010g/100mL. If these precision requirements are not met, the sample(s) must be reanalyzed.

4.2.7.11.5 **High Ethanol Values**

When an elevated ethanol value is obtained, appropriate calibrators must bracket the value. When necessary additional dilutions must be made. The dilution factor is incorporated into final calculations.

4.2.7.12 Reporting of Results

4.2.7.12.1 **Uncertainty**

4.2.7.12.1.1 Due to the uncertainty of measurement associated with any quantitative measurement, uncertainty values will be continually monitored through the evaluation of proficiency testing data.

4.2.7.12.2 **Breath Testing Solutions**

Provide results to the Breath Testing Program Manager for evaluation.

4.2.7.12.3 **Alcohol Beverages**

4.2.7.12.3.1 To obtain the ethanol concentration value the mean results of analysis must be multiplied by the dilution factor. This will provide the ethanol concentration in g/100cc (weight per volume (w/v) percent).

4.2.7.12.3.2 For volume per volume (v/v) value divide w/v value by 0.79.

4.2.7.12.3.3 Value must be reported as both w/v and v/v percent. The mean value must be truncated and reported as a whole number.

4.2.7.12.4 **Unknown Liquids and "Serum" - Ethanol**

4.2.7.12.4.1 Ethanol results must be reported concentration in g/100cc and/or weight per volume (w/v) percent depending on the sample history.

4.2.7.12.4.2 When dilution is necessary the mean results of analysis must be multiplied by the dilution factor.

4.2.7.12.5 **Unknown Liquids and "Serum" - Other Volatiles**

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

4.2.8 ANALYSIS DOCUMENTATION

4.2.8.1 Controls and standards are to be included in individual case files and/or a packet for the batch of samples is prepared.

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

4.2.8.3 When necessary, a copy of the control and standard printouts can be prepared from the centrally stored document.

4.2.9 QUALITY ASSURANCE

4.2.9.1 General

4.2.9.1.1 Samples are to be refrigerated while at the laboratory.

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

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

4.2.9.1.4 Refer to manufacturer manuals for maintenance procedures intended for the following tasks.

Gas Chromatograph

Task	*Indication*
Replace FID Jet	▪ Failure to ignite ▪ No signal ▪ Noisy signal
Replace O-Ring In The FID Colle	▪ Brittle or broken ▪ Noisy signal
Clean The FID Collector And Ca	▪ Noisy signal

Headspace Analyzer

Task	*Indication*
Replace Sampling Nee	▪ If damaged
Replace Needle Seal Assembly	▪ Check ~ every 2500 injecti
Replace O-Ring Seals	▪ Excessive carrier gas use ▪ May be required ~every 50 injections ▪ Upon inspection of needle seal, only O-ring may nee to be replaced. ▪ Retention time shifts

4.2.9.2 Quality Control Requirements Per Analysis Run

4.2.9.2.1 An internal standard blank must follow the highest ethanol calibrator.

4.2.9.2.2 For the analysis of simulator solutions, a minimum of two bottles of particular lot of simulator solution must be sampled.

4.2.9.2.3 For an analysis run which involves the quantitation of ethanol, two aqueous controls of the same ethanol concentration/level must be run per batch of 10 samples (20 vials). For each additional 10 samples, a minimum of one additional aqueous ethanol control must be run.

4.2.9.2.4 An aqueous control containing ethanol with or without other volatiles substances meets the "per run" requirement as described in 4.2.9.2.3.

4.2.9.2.5 In an analysis run which involves the qualitative identification of volatiles other than ethanol a multicomponent mixture, and/or a single component

aqueous standard containing each volatile to be identified, must be run.

4.2.9.3 Periodic Quality Control Requirements

4.2.9.3.1 The aqueous control concentration(s) must be varied periodically.

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

4.2.9.4 Monitoring of Quality Control Values

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

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

4.2.10 AUTHENTICATION OF REFERENCE MATERIALS

4.2.10.1 Aqueous Ethanol/Volatile Standards

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

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

4.2.10.1.3 New lots of Ethanol/Volatile standards must be included in duplicate in a minimum of one analysis run prior to official use.

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

4.2.10.1.5 **Ethanol**

The Certificate of Analysis for an aqueous ethanol calibration standard together with a comparison of relative retention time and quantitation data, against existing calibrators, will serve as the 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 their target value.

4.2.10.1.6 **Volatile Standards**
For standards (acetone, ethanol, methanol, isopropanol, ...) 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.

4.2.10.2 Commercially Obtained Aqueous Volatile Mixtures

4.2.10.2.1 *Certificate of Analysis* will be stored centrally.

4.2.10.2.2 The Certificate of Analysis for an aqueous mixed volatile standard along with a comparison to data from previous runs will serve as the qualitative authentication 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.

4.2.10.2.3 Refer to Certificate of Analysis for purity information.

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

4.2.11 REFERENCES

4.2.11.1 Stafford, D.T., Chromatography. in: Principles of Forensic Toxicology, edited by Barry Levin, pp. 93-101, 103-114, AACC Press, 1999.

4.2.11.2 Levine, B., Alcohol. in: Principles of Forensic Toxicology, edited by Barry Levin, pp. 170-184, AACC Press, 1999.

4.2.11.3 Caplan, Y.H., The Determination of Alcohol in Blood and Breath. in: Forensic Science Handbook, edited by Richard Saferstein, pp. 594-648, Prentice-Hall New Jersey, 1982.

4.2.11.4 Julien,R.M., Central Nervous System Depressants: Alcohol and the Inhalants of Abuse, in: Primer of Drug Action, pp. 64-92, Freeman-New York, 1998.

4.2.11.5 Saker, E.G., Screening and Quantitation by Head Space Technique of Some of the Vapors Most Commonly Found in Forensic Toxicology, in: Current Approaches in Forensic Toxicology, Chapter 11, SOFT Meeting, 1994.

4.2.11.6. Perrine, D.M., Depressants: Alcohol, Benzodiazepines, Barbiturates, in: The Chemistry of Mind-Altering Drugs, pp. 113-129, ACS, Washington, DC, 1996.

4.2.11.7 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.

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

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

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

Revision History

Section Four

Analysis of Alcohol and Common Volatile Solvents

4.2 Quantitative Analysis of Ethanol Containing Solutions

Revision #	Issue Date	Revisions
1	01-03-03	Original issue in SOP format
2	05-03-04	Clarifications, incorporation of serum and other toxicology unknown solutions, added volatiles other than ethanol. Validation issues covered when SOP 4.1 was validated for "other volatiles" therefore no validation necessary.
3	05-07-2007	Updated QA measures, nomenclature and formatting.