Idaho State Police
Forensic Services
Toxicology Section

Section Four
Analysis of Alcohol and Common Volatile Solvents

4.2 Quantitative Analysis of Ethanol Containing Solutions

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1	01-03-03	Original issue in SOP format

Approval

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4.2.1 BACKGROUND

The need to establish the ethyl alcohol concentration 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), and the need for verification of the ethyl concentration of simulator solutions used for breath testing instruments (IDAPA 11.03.01).

4.2.2 PRINCIPLE

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

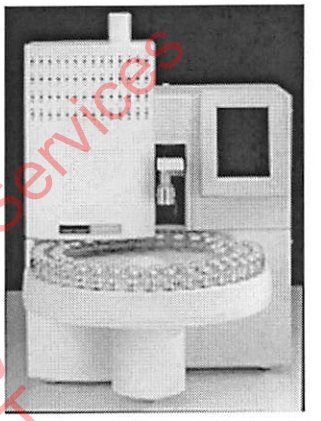

Figure 3. HS-110

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

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

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

4.2.3.7 Glassware

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

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

4.2.4 CONTROLS AND CALIBRATORS

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

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

4.2.5 REAGENTS

4.2.5.1 1-Propanol (Acros/Fisher Scientific # 23207-0010, #A996-1 or equivalent)

4.2.5.2 Acetone (Fisher #A929-1 or equivalent)

4.2.5.3 Acetaldehyde (Fisher #01004-250 or equivalent)

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

4.2.5.5 Methanol (Fisher #A454-1 or equivalent)

4.2.5.6 Ammonium Sulfate (Fisher #A702-500 or equivalent)
4.2.5.7 Sodium Fluoride (Fisher #S299-500 or equivalent)

4.2.6 SAFETY CONCERNS

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

4.2.7 REAGENT PREPARATION

Record the preparation of all reagents on reagent log.

4.2.7.1 Internal Standard Solution

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

4.2.7.1.1 1.0M $(NH_4)_2SO_4$

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

4.2.7.1.2 0.03g/dL 1-propanol in 1.0M $(NH_4)_2SO_4$

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

4.2.7.1.3 *Solution is stable for 3 months.*

4.2.7.2 Volatile Standard Mix Solution

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

4.2.7.2.2 Add the following volatiles, as indicated:

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

4.2.7.2.3 QS to 250-mL.

4.2.7.2.4 *Solution is stable for 1 year.*

4.2.8 ANALYSIS PROCEDURE

4.2.8.1 General

4.2.8.1.1 Unknown solutions can be included as part of a routine toxicology alcohol analysis run.

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

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

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

4.2.8.2 Quality Control

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

4.2.8.2.2 A minimum of two bottles of particular lot of simulator solutions should be sampled.

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

4.2.8.2.4 An aqueous control sample must be run after every 10 case samples. A minimum of two aqueous controls must be run per batch of samples.

4.2.8.2.5 Refer to package insert for manufacturer alcohol control ranges.

4.2.8.2.6 Values obtained from aqueous control samples must agree ± 10% of their target values.

4.2.8.2.7 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.

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

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

4.2.8.3 Pipetter/Dilutor Set-up

4.2.8.3.1 Switch on power.

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

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

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

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

4.2.8.4 Preparation of Blanks and Mixed Standard

4.2.8.4.1 Water Blank

4.2.8.4.1.1 Label test vial with *water blank.*

4.2.8.4.1.2 Add 2000µL DI water to labeled test tube.

4.2.8.4.1.3 Seal **immediately** with crimp cap.

4.2.8.4.2 Internal Standard Blank

4.2.8.4.2.1 Label test vial with *ISTD blank.*

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

4.2.8.4.2.3 Seal **immediately** with crimp cap.

4.2.8.4.3 Aqueous Controls

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

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

4.2.8.4.3.3 Seal **immediately** with crimp cap.

4.2.8.4.4 Mixed Other Volatiles Solution

4.2.8.4.4.2 Label test vial with *mixed volatiles.*

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

4.2.8.4.4.3 Seal **immediately** with crimp cap.

4.2.8.5 Preparation Calibration Standards

4.2.8.5.1 Label vials for standards.

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

4.2.8.5.3 Seal **immediately** with crimp cap.

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

4.2.8.6 Initial Processing of Specimens

4.2.8.6.1 After inspecting and documenting the condition of seals, remove sample(s) container from outer packaging and place laboratory number on each sample.

4.2.8.7 Preparation of Samples for Analysis

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

4.2.8.7.2 Dilute alcoholic beverages are necessary. The sample should 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. Breath testing simulator solutions do not require dilution.

4.2.8.8 Addition of sample to headspace vials.

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

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

4.2.8.9 Preparation for Run

4.2.8.9.1 Open **Sequence Editor**

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

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

4.2.8.9.4 Active headspace sampler

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

4.2.8.10 Gas Chromatography Parameters

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

4.2.8.11 Calibration

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

4.2.8.11.2 The least squares line resulting from the analysis of the ethanol calibrators must have a coefficient of correlation of ≥0.995.

4.2.8.12 Acceptance Criteria

4.2.8.12.1 **Accuracy**

4.2.8.12.1.1 Qualitative

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

4.2.8.12.1.2 Quantitative

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

4.2.8.12.2 **Precision**

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.

4.2.8.13 Reporting of Results

4.2.8.13.1 **Breath Testing Solutions**

4.2.8.13.1.1 Provide results to the Breath Testing Program Manager for evaluation.

4.2.8.13.2 **Alcohol Beverages**

4.2.8.13.2.1 To obtain the ethanol concentration value the mean results of analysis should be multiplied by the dilution

factor. This will provide the ethanol concentration in g/100cc or weight per volume (w/v) percent.

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

4.2.8.13.2.3 Value should be reported as both w/v and v/v percent to 1 significant figure.

4.2.9 QUALITY ASSURANCE

4.2.9.1 Samples are to be refrigerated while at the laboratory.

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

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

4.2.9.4 Refer to toxicology manual section 5.3.2 for GC-HS maintenance requirements.

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

4.2.10 ANALYSIS DOCUMENTATION

4.2.10.1 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.

4.2.10.2 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.

4.2.11 REFERENCES

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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.

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