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

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Quality Manager

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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).

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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 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 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 1-propanol internal standard. These solution samples can be included as part of a toxicology alcohol determination run utilizing Analytical Method 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 Agilent 7890A Gas Chromatograph (GC) configured with a Flame Ionization Detector (FID) (Figure 2).

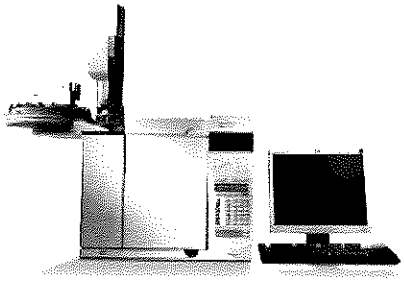

Figure 2. Gas Chromatograph

4.2.3.2 Agilent G1888 Headspace Sampler (Figure 3).

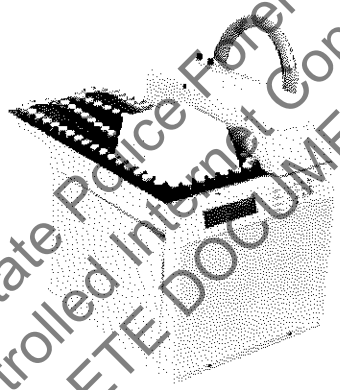

Figure 3. Headspace Analyzer

4.2.3.3 Columns

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

4.2.3.3.2 Restek Rtx®-BAC2 (#18002: 30 meter X 0.32mm ID, 1.2 µm FT or equivalent column)

4.2.3.4 Headspace (HS) vials and Closures

4.2.3.5 Hand Crimper or Bench Top Crimper

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

4.2.4 REAGENTS

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

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4.2.4.1 Distilled/Deionized water (free from volatiles of interest)
4.2.4.2 1-Propanol/n-Propanol (≥99%)
4.2.4.3 Acetone (≥99%)
4.2.4.4 Acetaldehyde (≥99%)
4.2.4.5 Isopropanol/2-Propanol (≥99%)
4.2.4.6 Methanol (≥99%)
4.2.4.7 Toluene (≥99%)
4.2.4.8 Ammonium Sulfate (Certified ACS Grade)

4.2.5 REFERENCE MATERIAL

Record the preparation of all solutions on reagent log.

4.2.5.1 Ethanol Aqueous Reference Material

4.2.5.1.1 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.

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

4.2.5.2 Multicomponent Volatile Aqueous Solutions

Multicomponent solutions may be purchased or prepared as indicated below.

4.2.5.2.1 **Commercially Obtained Multicomponent Solution**

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

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

4.2.5.2.1.3 When the multicomponent solution contains quantitative amounts of ethanol, it may simultaneously serve as an aqueous ethanol control if the GC oven

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temperature program provides for baseline separation of all components.

4.2.5.2.2 **Prepared Mixed Volatile Solution**

4.2.5.2.2.1 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. Solution is stable for 1 year when stored under refrigeration.

4.2.5.2.2.2 Additional volatiles of interest may be added to the mixed volatile solution. The GC oven temperature conditions must provide for baseline separation from other components in the mixture.

4.2.5.3 Internal Standard Solution

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

4.2.5.3.1 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.

4.2.5.3.2 Solution is stable for 1 month when stored at room temperature. Other volumes of internal standard may be prepared as needed.

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4.2.6 SAFETY CONCERNS

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

4.2.7 QUALITY ASSURANCE

4.2.7.1 General

4.2.7.1.1 While at the laboratory samples for volatiles testing are to be stored under refrigeration.

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

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

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

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

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

4.2.7.1.6 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.

4.2.7.2 Calibration Curve/Table Requirements

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

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

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

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

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

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

4.2.7.2.4 Each ethanol calibrator may have more than one replicate.

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

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

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

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

4.2.7.2.7 Once established, analysts not involved in establishing the calibration curve/table may use the established calibration.

4.2.7.2.8 An analysis run may include case samples prepared by more than one analyst.

4.2.7.3 Per Analysis Run Control Requirements

4.2.7.3.1 **Calibration Run**

4.2.7.3.1.1 An internal standard blank should follow the last (highest) ethanol calibrator.

4.2.7.3.1.2 A water blank may be included in each calibration run.

4.2.7.3.1.3 For up to 10 samples (20 vials), an analysis run must include either a high or low control in duplicate before proceeding with additional samples.

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4.2.7.3.1.4 For analysis run consisting of more than 10 samples (20 vials), a minimum of one control must be run with each additional 10 samples (20 vials).

4.2.7.3.1.5 A blood or aqueous control containing ethanol, with or without other volatile substances, meets the "per run" requirement.

4.2.7.3.1.6 Each calibration run must include either an aqueous or blood multicomponent volatile mix.

4.2.7.3.1.7 A commercially obtained quantitative multicomponent volatile mix may be used as both an aqueous ethanol control and a multicomponent mixture.

4.2.7.3.2 **Additional Runs with Existing Calibration Table**

4.2.7.3.2.1 The same batch of internal standard used in the calibration run must be used for additional runs within the two-week period.

4.2.7.3.2.2 An internal standard blank should follow the analysis of the high blood control.

4.2.7.3.2.3 The analysis run must include both high and low blood and/or aqueous controls in duplicate. If blood volatiles (analytical method 4.1) are a portion of the run, at least one set of duplicates must be blood controls.

4.2.7.3.2.4 When the analysis exceeds 10 samples (20 vials), a minimum of one blood or aqueous control must be run with each additional 10 samples (20 vials).

4.2.7.3.2.5 A blood or aqueous control containing ethanol, with or without other volatile substances, meets the "per run" requirement.

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4.2.7.3.2.6 Additional aqueous controls can be included at the discretion of the analyst.

4.2.7.4 Uncertainty of Measurement

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

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

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

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4.2.8 PRE-RUN SAMPLE PREPARATION

4.2.8.1 Inspect and document the condition of outer seals.

4.2.8.2 Removed sample container(s) from packaging, inspect and document the condition of container seal and label container with laboratory number.

4.2.8.3 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.

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

4.2.9 PRE-RUN PIPETTER/DILUTOR SET-UP

4.2.9.1 Switch on power.

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

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

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

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

4.2.10 ANALYSIS PROCEDURE

4.2.10.1 General

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

4.2.10.1.2 Label headspace vials for case samples, calibrators and controls.

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

4.2.10.1.5 Lot numbers for calibrators and controls must be recorded on task sheet, in sequence, and/or solution preparation log.

4.2.10.2 Headspace Vial Preparation

4.2.10.2.1 Use Pipetter/Dilutor dispense 250µL of case samples, controls and calibrator solutions along with 2000µL of internal standard (ISTD) into labeled headspace vial.

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

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

4.2.10.3 Instrument Run Preparation

4.2.10.3.1 Open **Sequence Table.** It is recommended that each analyst prepare and use their own sequence. This reduces the possibility of the sequence being modified without their knowledge.

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

4.2.10.3.3 Load samples, calibrators, blank and controls onto the headspace sampler carousel rack as noted in the sequence table.

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

4.2.10.4 Instrument Parameters

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

4.2.10.4.2 The method must be set up such that all samples (casework, calibrators, and controls) are quantitated to a minimum of three decimal places (0.000).

4.2.10.4.3 Analysis method printouts must be stored centrally (hardcopy and/or electronically).

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4.2.11 ACCEPTANCE CRITERIA

4.2.11.1 Qualitative Accuracy Criteria

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

4.2.11.2 Quantitative Accuracy Criteria

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

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

control TABS are used to calculate the mean values for calibrator and control samples.

4.2.11.2.3 The quantitative ethanol results for a batch of samples can be accepted if the values obtained for control samples fall within ±10% of target value. Target values are determined as described in Analytical Method 4.1, sections 4.1.14.1.5 and 4.1.14.1.6.

4.2.11.3 Out of Range Control(s)

4.2.11.3.1 **Single Value**

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

4.2.11.3.1.2 The analysis run may only be accepted if the anomaly is in excess of the required number of controls as described in section 4.2.7.3, Per Analysis Run Control Requirements.

4.2.11.3.2 **Two or More Values**

4.2.11.3.2.1 When more than one control value falls outside of the either the required concentration or precision range, the casework samples following the non-conforming controls must be reanalyzed.

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

4.2.11.4 Column Precision Criteria

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

4.2.11.4.2 If the instrument's MACRO is not used, a copy of the I:\ drive "Volatiles Determination Casefile Worksheet" (or equivalent) can be used. The casefile TAB can be

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used to calculate the column precision. The calibrator and control TABS are used to calculate the column precision for calibrator and control samples.

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

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

4.2.11.5 Quantitative Replicate Precision Criteria

4.2.11.5.1 When more than one replicate for a case, calibrator or control sample, is analyzed, calculate the mean value (columns 1 and 2) for each sample with volatiles analysis spreadsheet form.

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

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

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

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

4.2.11.6 High Ethanol Values

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

4.2.12 REPORTING OF RESULTS

4.2.12.1 Breath Testing Solutions

Provide results to the Breath Testing Program Discipline Leader for evaluation.

4.2.12.2 Alcohol Beverages

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

4.2.12.2.2 The mean ethanol value is calculated as described in section 4.2.11.2.

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

4.2.12.2.4 To obtain the ethanol concentration value the adjusted ethanol concentration results is multiplied by the dilution factor. This will provide the ethanol concentration in g/100cc (weight per volume (w/v) percent).

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

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

4.2.12.3 Unknown Liquids and "Serum" - Ethanol

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

4.2.12.3.2 The mean ethanol value is calculated as described in section 4.2.11.2.

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

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

4.2.12.4 Unknown Liquids and "Serum" - Other Volatiles

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

4.2.12.5 Reporting of Qualitative Volatiles Results

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

4.2.13 ANALYSIS DOCUMENTATION

4.2.13.1 Volatiles Analysis Forms

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

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

4.2.13.1.3 The case sample spreadsheet must contain the current analysis parameters.

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

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

4.2.13.2 Quality Assurance Data

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

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

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

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4.2.14 AUTHENTICATION OF REFERENCE MATERIALS

Refer to Analytical Method 4.1.

4.2.15 REFERENCES

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Revision History

Section Four

Analysis of Alcohol and Common Volatile Solvents

4.2 Quantitative Analysis of Ethanol Containing Solutions

Revision #	Issue Date	Revisions
1	01-03-2003	Original issue in SOP format
2	05-03-2004	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.
4	08-20-2008	Updated for new instrumentation. Deviation in place prior to this date. Authentication process referenced to Analytical Method 4.1. Added uncertainty language.

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