2.0 Analysis of Solutions Containing Ethanol and Common Volatiles

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).

2.2 SCOPE

This method describes the analysis of solutions said to contain a specified amount of ethyl alcohol and 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. These solution samples can be included as part of a toxicology alcohol determination run utilizing Volatiles Analytical Method 1.0 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 included in the analysis run.

Note that in this analytical method the terms calibrator and calibration are not used in the ISO/IEC 17025:2005 sense. The words *calibrator* and *calibration* are used to coincide with the terminology in instrument software and manufacturer manuals. The manufacturer's term *calibrator* refers to what is considered by ISP-FS as aqueous reference material that has a certified concentration of ethanol present. This aqueous reference material is used to establish a three to six point *calibration curve/table* to establish a response factor between instrument response and reference material concentration.

2.3 EQUIPMENT

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

Figure 2. Gas Chromatograph

2.3.2 Agilent G1888 Headspace Sampler (Figure 3).

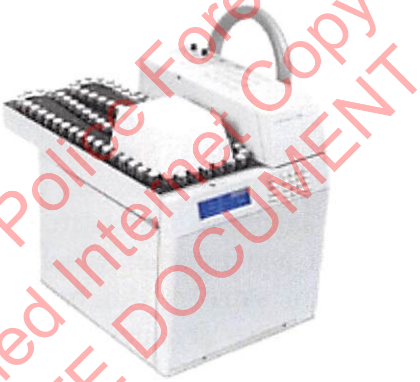

Figure 3. Headspace Analyzer

2.3.3 Columns
  2.3.3.1 Restek Rtx®-BAC1 (#18003: 30 meter X 0.32mm inner diameter (ID), 1.8µm film thickness (FT) or equivalent column)
  2.3.3.2 Restek Rtx®-BAC2 (#18002: 30 meter X 0.32mm ID, 1.2 µm FT or equivalent column)
2.3.4 Headspace (HS) vials and Closures
2.3.5 Hand Crimper or Bench Top Crimper
2.3.6 Semi-Automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250µL and 2000µL, respectively.

2.4 REAGENTS

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

2.4.1 Distilled/Deionized water (free from volatiles of interest)
2.4.2 Ammonium Sulfate (Certified ACS Grade)

2.5 REFERENCE MATERIAL

Record the preparation of all solutions on reagent log.

2.5.1 Ethanol Aqueous Reference Material

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.

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 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***.

2.5.2 Multicomponent Volatile Aqueous Solutions

Multicomponent solutions may be purchased or prepared as indicated below.

2.5.2.1 **Commercially Obtained Multicomponent Solution**

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

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

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 temperature program provides for baseline separation of all components.

2.5.2.2 **Prepared Mixed Volatile Reference Solution**

Reference material used to prepare mixed volatile solution must be ≥99%. A multi-component mixture and/or a single component solution may be used for identification purposes.

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. Record preparation on reagent log. Solution is stable indefinitely when stored under refrigeration.

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

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

2.5.3 Internal Standard Solution

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

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.

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.

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.*

2.7 QUALITY ASSURANCE

2.7.1 General

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

2.7.1.2 The syringes on the Pipetter/Dilutor must be checked for accuracy and precision. Refer to Volatiles Analytical Methods 3.0 and 4.0 for pipette intermediate check and calibration requirements and options.

2.7.1.3 Refer to Volatiles Analytical Method 5.0 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.

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

2.7.1.5 Current source and lot number of controls and reference material must be documented.

2.7.1.6 If ethanol is not the analyte in question, a calibration curve for ethanol need not be established.

2.7.1.7 If samples prepared by this method are analyzed on the HSA-GC-FID along with samples prepared by Volatiles Analytical Method 1.0, duplication of required controls is not necessary.

2.7.1.8 Refer to Volatiles Analytical Method 10.0 for quantitative ethanol reporting confidence interval/uncertainty.

2.7.1.9 Refer to Analytical Methods 8.0 and 9.0 for authentication requirements for volatiles reference material and matrix controls.

2.7.1.10 Each analysis run must contain an internal standard blank.

2.7.2 Calibration Curve/Table Requirements: Ethanol Quantitation

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

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

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

2.7.2.1.3 Calibration table may be established in a separate sequence just prior to sequence containing case samples.

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

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.

2.7.2.4 Each ethanol calibrator may have more than one replicate.

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".

2.7.2.5 If data from a calibrator is not usable, the remaining data can be used to establish the response factor provided specifications listed in 2.7.2.1 and 2.7.2.3 are met.

2.7.2.6 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.

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

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

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

2.7.3 Per Analysis Run Control Requirements: Ethanol Quantitation

2.7.3.1 **Initial Run with Calibration Curve**

For a run with a newly established calibration curve, an ethanol containing control, in duplicate, must be included with the first ten samples (20 vials) before proceeding with the run.

2.7.3.2 **Additional Runs with Existing Calibration Curve**

For analysis runs utilizing an existing calibration curve, a low and high ethanol-containing control, in duplicate, must be included with the first 10 samples before proceeding with additional samples.

2.7.3.3 **All Quantitative Analysis Runs**

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

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

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

2.7.4 Per Analysis Run Control Requirements: Qualitative Analysis Only

2.7.4.1 A multicomponent mixture of previously characterized volatiles of interest must be included in each analysis run.

2.7.4.2 In addition, single-component controls can be analyzed as considered necessary.

2.8 PRE-RUN SAMPLE PREPARATION

2.8.1 Inspect and document the condition of outer seals.

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

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.

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

2.9 ANALYSIS PROCEDURE

2.9.1 General

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

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

2.9.1.3 Prepare pipetter/dilutor for use.

2.9.1.4 All case samples must be analyzed in duplicate. Calibrators may be run in duplicate. Refer to sections 2.7.3 and 2.7.4 for Positive Control replicate requirements.

2.9.2 Headspace Vial Preparation

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

2.9.2.2 For internal standard blank, use Pipetter/Dilutor to dispense 250µL of DI water along with 2000µL of internal standard (ISTD) into labeled headspace vial and seal.

2.9.3 Instrument Run Preparation

2.9.3.1 Open **Sequence Table.** It is recommended that each analyst create, not share, a Sequence Table. This reduces the possibility of the Sequence Table being modified without their knowledge. If a Sequence Table is shared, each analyst must inspect the Sequence prior to analysis.

2.9.3.2 Into Sequence log table, enter the sample case numbers and information for appropriate QA samples (ethanol calibrators, other volatiles/mix, blanks and controls).

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

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

2.9.4 Instrument Parameters

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

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

2.10 CRITERIA FOR ACCEPTANCE OF DATA

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

2.10.2 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.

2.10.3 All results obtained from samples bracketed by conforming controls are acceptable for use.

2.10.4 When a control value falls outside of required qualitative, quantitative and/or precision acceptance criteria, the 10 casework samples preceding and following the non-conforming control(s) must be reanalyzed. If only the quantitative criterion is not met, this reanalysis requirement does not apply to samples that are being processed for the qualitative presence of volatiles other than ethanol.

2.10.5 Qualitative Accuracy Criteria

The qualitative presence of ethanol, or other volatile substances, can be established if the retention time for a specimen is within ±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.

2.10.6 Quantitative Accuracy Criteria

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 for blood controls are determined as described in Analytical Method 8.0.

2.10.7 Column Precision Criteria

2.10.7.1 The ethanol values obtained from column 1 and 2 must agree within 0.0150g/100cc.

2.10.7.2 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.

2.10.8 Quantitative Replicate Precision Criteria

2.10.8.1 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

2.10.8.2 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.

2.10.8.3 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.

2.11 REPORTING OF RESULTS

2.11.1 General

The three decimal place truncated mean ethanol value, as determined by this method, will be reported along with the ± 95% confidence interval range in the form of Coefficient of Variation (CV%). The determination of the confidence interval is addressed in Volatiles Analytical Method 10.0.

2.11.2 Breath Testing Solutions

Provide results to Discipline Leader for evaluation.

2.11.3 Alcohol Beverages

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

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

2.11.3.3 Value must be reported as both w/v and v/v percent. The mean value must be truncated to three decimal places and reported as a whole number ±CV%.

2.11.4 Unknown Liquids and "Serum" - Ethanol

2.11.4.1 Report ethanol concentration in g/100cc and/or weight per volume (w/v) percent, depending on the sample history.

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

2.11.4.3 When reporting as g/100cc, report mean ethanol concentration, truncated to three decimal places (0.000), as grams of ethanol per 100cc of liquid ±CV%.

2.11.5 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.

2.12 ANALYSIS DOCUMENTATION

2.12.1 Volatiles Analysis Forms

Required spreadsheet form can be located under:
I:\International Management System\Toxicology\Toxicology Forms

2.12.2 Quality Assurance Data

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

2.12.2.2 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.

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

2.13 AUTHENTICATION OF REFERENCE MATERIALS AND CONTROLS

Refer to Analytical Methods 8.0 and 9.0.

2.14 REFERENCES

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2.14.4 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.

2.14.5 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.

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

2.14.7 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.

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

2.14.9 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.

2.14.10 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

2.0 Analysis of Solutions Containing Ethanol and Common Volatiles

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.
5	09-07-2009	Uncertainty approach updated and referenced to AM 5.13. Clarified QA requirements. Clarified that no ethanol calibration table/curve is necessary when performing qualitative analysis for other volatiles.
0	01-20-2011	Initial version as 1.0, split from toxicology discipline analytical methods. Formerly AM 4.2. Clarified QA requirements.

From: Johnston, Jeremy
Sent: Friday, August 19, 2011 1:04 PM
To: Gamette, Matthew
Subject: rescind

AM 2.0, 3.0, 5.0 and 9.0 are no longer in use or have been incorporated into other AM's and can be archived as no longer in use.

AM 2.0 has been incorporated into AM 1.0
AM 9.0 has been incorporate into AM 8.0
AM 5.0 is redundant to the requirements of Tox or Drug AM's and referenced as such.
AM 3.0 is redundant with Tox AM's

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