Colorado Department of Public Health and Environment

Laboratory Services Division
Toxicology Laboratory

Ethanol, Headspace GC-FID, Blood/Urine/Aqueous

Revision 4

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TITLE

Ethanol, Headspace GC-FID, Blood/Urine/Aqueous

REFERENCES

Dubowski, Kurt M. Manual For Analysis of Ethanol in Biological Liquids. U.S. Department of Transportation, DOT-TSC-NHTSA-76-4 (HS 802 208)

Handbook of Chemistry and Physics, 56th Edition 1975-1976, CRC Press, 18901 Cranwood Parkway, Cleveland, Ohio 44128

Committee Handbook, National Safety Council – Committee on Alcohol and Other Drugs, 1992. "A Model Program For the Control of Alcohol For Traffic Safety."

Rules and Regulations of the Colorado Board of Health Relating to Test For Alcohol and Other Drugs, 5-CCR-1005-2, Part 1.3.1.

Davidsohn, Israel and Bernard, John. Todd-Sanford Clinical Diagnosis, 15th edition. Philadelphia: Saunders Company, 1974.

"Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's (ACS) Department of Governmental Regulations and Science Policy, 1155 16th Street NW, Washington D.C. 20036, (202) 872-4477.

METHOD

Analysis is performed by headspace gas chromatography with a flame ionization detector.

PRINCIPLE

A blood, urine, or aqueous specimen and internal standard are quantitatively pipetted into a 20 mL headspace vial and sealed. The vial is heated and pressurized forcing ethanol and other volatiles into the headspace. The gas in the headspace is then quantitatively analyzed by gas chromatography with a Flame Ionization Detector (FID).

SAMPLES

This procedure is intended to analyze human blood or urine, as well as aqueous samples and standards.

Note: This procedure is intended for human blood samples preserved with sodium fluoride and potassium oxalate (gray top tubes). Human blood in other tubes may be analyzed, but the type of tube must be noted on the report. The purpose of the sodium fluoride is to prevent bacteria growth and the potassium oxalate is to prevent clotting.

SAFETY

Use routine precautions found in the Chemical Hygiene Plan (Appendix I – Safety Manual) when working in the laboratory. Follow the Bloodborne Pathogens Exposure Control Plan (Appendix G – Safety Manual), when working with biological fluids or tissues. Read all Material Safety Data Sheets before handling unfamiliar reagents.

EQUIPMENT

1. Agilent Gas Chromatograph with dual Flame Ionization Detectors (FID), or equivalent using dual columns with different retention times such as the Restek Rtx-BAC1 and Rtx-BAC2, or the J&W DB-Alc1 and DB-Alc2, or equivalent

2. Headspace auto-sampler such as the Tekmar HT-3 or equivalent

3. HP Chemstation, or equivalent for data processing

4. Volumetric pipettes (Class A required for standard preparation)

5. Adjustable volume pipettes, and or specific volume pipettes (Finnpipettes or equivalent)

6. Stir plate and magnetic stir bars

7. Volumetric flasks, Class A, various sizes

8. Beakers

9. Headspace vials, 20 mL

10. Vortex mixer

11. Serum vials, 10 mL

12. Caps and stoppers for headspace and serum vials

13. Headspace vial cap-crimper

14. Benchcoat lab bench covering, or equivalent

15. Disposable pipette tips
    Note: Tips are used for one standard or specimen only and are then disposed of in Biohazard bags for autoclaving.

16. Secure refrigerators for sample storage

Note: A secure refrigerator either has a lock or is in a secure lab room.

17. Specimen storage racks and containers

REAGENTS

Reagent grade chemicals shall be used in all tests unless otherwise indicated (e.g. 99.99% n-propanol is required). It is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

Chemicals

1. 200 proof ethyl alcohol, density 0.7893 (also known as ethanol or EtOH), or National Institute of Standards and Technology (NIST) ethanol standard material
2. Acetone
3. N-Propanol
4. Methanol (MeOH)
5. Isopropanol
6. Sodium fluoride
7. Sodium carbonate, anhydrous
8. Bleach (sodium hypochlorite)
9. Deionized (DI) water

Gases

Gases may be used from either gas generators or commercially purchased cylinders (tanks). If generators are used, the gases must meet or exceed the quality of the tanks listed below. All gases in use are to be monitored each working day. If generators are in use they should be checked to ensure they are functioning properly. If gas tanks are in use, pressures should be recorded on the maintenance log for the corresponding instrument. When tank pressures fall below 500 psi, they are to be replaced and leak checked. Following replacement, the new tank is to be listed on the gas leak check log and the date it was put into service.

1. Compressed Air Gas Tank (UN1002)

2. Helium Gas Tank (UN1046, UHP Zero Grade)

3. Hydrogen Gas Tank (UN1049)

4. Nitrogen Gas Tank - ultra high purity

Standards

Cerillilant or Restek aqueous ethanol standards, or equivalent (for calibration)

- 0.010% Ethanol
- 0.100% Ethanol
- 0.200% Ethanol
- 0.400% Ethanol

Controls

Cerilliant or Restek aqueous ethanol controls, or equivalent (various concentrations)

- 0.050% Ethanol
- 0.080% Ethanol
- 0.100% Ethanol
- 0.150% Ethanol
- 0.200% Ethanol
- 0.300% Ethanol

Internal Standard

0.080% N-Propanol

Matrix Solutions

1. Defibrinated sheep's blood

2. Negative urine

3. D.I. water

REAGENT/STANDARD PREPARATION

Notes:

- All "working" chemical solutions, mixtures, or dilutions shall be labeled with the following information; chemical name, concentration, date prepared, analyst's initials, special storage instructions, and expiration date.
- Calibration standards and controls (Restek, Cerilliant or equivalent) are NIST

traceable aqueous ethanol standards and are stored in a secure refrigerator at less than 8 degrees Centigrade.

- Storage of blood alcohol negative standards: Defibrinated sheep's blood which has been supplemented with sodium fluoride to a concentration of 1% is placed into a properly labeled vial, then cap and store the blood in a secure refrigerator at less than 8 degrees Centigrade. After opening, the standard can be used one week.

1. 0.08% n-propanol (internal standard): Use a volumetric pipet to dispense 1.0 milliliter (mL) of n-propanol (minimum 99.99% pure) into a 1-L volumetric flask that is partially filled with DI water. Dilute to 1.0 liter with DI water. Note: The solution must be at room temperature or the volume may be inaccurate. Mix well before using. Store at room temperature in a tightly capped, labeled Nalgene bottle.

2. Blank/Negative Blood Standard: Place an amount of NaF into the volume of blood to be used such that the resulting concentration is 1% w/v. Example: For 1 Liter of blood, 10g of NaF will be added. Mix well. Label and store tightly capped in a secure refrigerator. (Storage in 10 mL serum vials is preferred.) Always bring to room temperature before using. Note: The solution must be at room temperature or the volume may be inaccurate.

3. Mixed Volatile Solution: To make the Mixed Volatile Stock Solution: Use volumetric pipets to dispense the following amounts of reagents into a 100 mL volumetric flask that is partially filled with DI water. 1 mL MeOH, 1.5 mL EtOH, 0.5 mL 2-propanol (isopropanol), 0.5 mL n-propanol, and 0.5 mL acetone. Dilute to 100 mL with DI water. This is used for qualitative purposes only.

   To make the Mix Working Solution: Use a Finnpipette (or better) to dispense 20 mL of the Mixed Volatile Stock Solution into a 100 mL volumetric flask partially filled with DI water. Dilute to 100 mL with DI water. Store in a properly labeled bottle.

4. 10% Bleach: Mix 50 mL of bleach with 450 mL water. Properly label and store at room temperature. Make fresh at least weekly. This is used for cleaning only and measurements do not need to be precise.

PROCEDURE

Specimen Security and Chain of Custody

Accurate specimen identification is required at all times with zero exceptions. The analyst must assure accuracy of specimen identification at all times including before analysis, during analysis, after analysis, and during storage. Discrepancies must be noted and resolved before reporting results.

Note: It is preferred for the analyst to initial the Request for Analytical Services form after

confirming information matches and no discrepancies exist between the samples and paperwork prior to analysis.

Specimen Preparation

Note: Blood specimens normally are prepared for analysis in a Purifier Class II Biosafety Cabinet with the glass shield pulled down to the marked level to assure adequate air flow. Eye protection, latex (or equivalent) gloves, and lab coat should be worn. Benchcoat or equivalent should be placed on the cabinet or bench work surface. If specimens are prepared on an open lab bench, face protection also should be worn. Cutting tools and other sharp objects must be handled with care and stored in such a manner as to prevent cuts and scratches. Upon completion of specimen preparation, the work area is to be cleaned using 10 % bleach (prepared weekly). Assure no blood is left on working areas, tools, or equipment. The Biosafety cabinet should be closed and the ultraviolet light turned on for a minimum of 10 minutes.

All blood specimens are to be handled and stored as biohazardous material. Open all specimens with the tubes and containers pointed away from self and others. Dispose of blood-contaminated waste in biohazard waste bags and take them to be treated in the autoclave prior to disposal.

1. Standards, controls, and specimens are to be stored in a secure refrigerator at a temperature of less than 8 degrees Centigrade. The refrigerator is to be labeled as containing biohazardous material.

2. Allow all standards, controls, and specimens to come to room temperature before pipetting.

3. All standards, controls, and specimens are to be mixed on a platform shaker for a minimum of 5 minutes, or shall be manually inverted a minimum of 10 times.

4. Use a permanent marker to label 20 mL headspace vials with the standard/theoretical value, control value, or specimen identification.

5. Use Eppendorf, Finnpipette, or equivalent pipets to dispense volumes of aliquots into headspace vials.

   Mix Volatile Solution (for Blood, Urine and Aqueous analysis)
   2 mL reagent water
   0.5 mL working mix volatile solution

   Controls and specimens (for Blood, Urine and Aqueous analysis)
   1.0 mL DI water
   1.0 mL 0.08% n-propanol internal standard

0.5 mL control or specimen
Note: Use control values of 0.050, 0.080, 0.100, 0.150, 0.200, 0.300.

Calibration Standards
0.5 mL DI water
1.0 mL 0.08% n-propanol internal standard
0.5 mL Matrix solution (prepared negative blood, negative urine, or D.I. water)
0.5 mL NIST traceable aqueous ethanol standard (0.01%, 0.1%, 0.2%, 0.4%)

Note: Never use two different internal standards in one analytical run. Accurate dilutions may be used when needed. This includes when there is not enough specimen or when the value is above the highest calibration standard.

6. Blood and aqueous specimens are to be analyzed in duplicate, unless there is not enough sample. If only one aliquot is analyzed, note this on the requisition under comments. One aliquot of urine specimen will be analyzed following a positive screen result.

7. Cap and crimp all headspace vials.

8. Vortex each vial to assure adequate mixing. Avoid getting liquid in the upper vial or on the cap (set the vortexer to between 5 and 6).

9. Analyze these immediately or return to refrigerated storage.

10. If the blood analyzed was not in a gray top tube, the color of the tube cap used for analysis must be noted on the requisition. Note any other discrepancies found during analysis for any sample type.

Sample Storage

When specimen preparation is complete, samples are placed in a secure refrigerator for storage. Temperature is to be less than 8 degrees Centigrade. Temperature is to be checked once per regularly scheduled working day. The temperature is to be recorded and initialed on the appropriate form kept on the refrigerator.

Store samples in appropriate areas (bloods with bloods, urines with urines, aqueous with aqueous, etc.) in numerical (Lab ID number) order for a minimum of one year per the *Rules and Regulations*. Disposal of specimens will be recorded in the specimen disposal log. (Initial and date all disposals.) Some specimens must have the appropriate District Attorney's approval before disposal. These are stored separately with a copy of the letter requiring special storage.

Instrument Settings

1. Tekmar
   Make certain that the Tekmar is on and set for ethanol analysis. Enter the appropriate sequence.

2. Gases
   Generators should be monitored every working day. Gas tank pressure should be greater than 500 pounds. If at or below 500 pounds, change the tank. Fill in the appropriate log sheets.

3. Gas Chromatograph
   Make certain that the FID is on and that the instrument is set for the appropriate sample method.

4. Computer Controls
   Edit sample table to provide the day's list of standards, controls, and specimens. Save the table using "mmddyy.s" with the date being the same as in the sequence parameters.
   Print the sequence.

Analysis of Specimens

1. Place vials in the Tekmar carousel in the following order:

Position No.	Sample Identification
1	Mix
2	Negative
3	0.010% matrix standard
4	0.100% matrix standard
5	0.200% matrix standard
6	0.400% matrix standard
7	negative
8	control
9	control
10	control
11	specimens start here

Note: The first three controls may vary according to the analyst's disgression. Typically the order is 0.050%, 0.150%, and 0.300%.

2. Controls at the end of the run typically include a 0.050% control, a negative matrix, and a 0.300% control.

3. Controls are placed after every 10 specimen vials (5 subjects with duplicate aliquots) in the day's run. Control values should range from 0.000% ethanol to 0.300% ethanol.

DATA INTERPRETATION AND QUALITY CONTROL

1. Acceptance/rejection of standards and controls: Values within +/- 5% of the theoretical value are acceptable without further review. Values within +/- 10% of the theoretical value are acceptable when approved by experienced personnel. Values greater than +/- 10% of the theoretical value are acceptable only if justified by the supervisor or the supervisor's designee on an individual basis.

2. The calibration curve must be reviewed and have a correlation coefficient of 0.995 or better, if acceptable, the analytical run can be continued. If it is unacceptable, experienced personnel or the supervisor will determine necessary actions up to and including restarting the analysis from the beginning.

3. Values of less than 0.010% for blood and aqueous samples are to be reported as "none detected". Values of less than 0.040% for urine samples are to be reported as "none detected".

4. The two aliquots of a blood or aqueous sample should be within +/- 5 %. If they are not, the specimen should be reanalyzed. Note: QA officer or supervisor may approve up to 10 % variation.

5. Results of the two aliquots are averaged and rounded down. They are reported to three decimal places.

6. Urine samples are only analyzed once and are reported qualitatively as Positive or Negative.

7. All chromatographs should be reviewed for the possible presence of non-ethanol volatiles. Positive identification of other volatiles should be noted on the chromatograph, initialed and dated.

8. Review each chromatograph for the following: specimen identification, internal standard and ethanol peaks, other volatile peaks, internal standard peak area, and ethanol concentration.

9. Enter specimen identifications and ethanol concentrations on the Excel spreadsheet and determine the average value for each specimen.

10. Enter standard and control values in quality control spreadsheets.

11. Review the data spreadsheet for accuracy of specimen identifications, individual ethanol values, average ethanol values, and note specimens requiring reanalysis. All averages should be rounded down, not up.

12. Sign and date the Excel spreadsheet.

13. Enter ethanol values in the LITS computer and check the accuracy of data entry.

14. Print reports.

15. Review each report and assure accuracy of the report as compared to the Analytical Requisition and the analytical data. Assure discrepancies/comments are included.

16. All data and reports are to be fully and completely reviewed by a second person.

17. The reviewer is to sign and date the reports with the date of the review.

18. The QA officer and/or the supervisor are to sign the reports.

19. Copy and mail reports. The original requisition and a copy of the analytical report are to be filed in numerical order. A copy of the requisition, chain of custody evidence and the original analytical reports are to be mailed (or e-mailed) to the customer.

20. All quality assurance review and actions listed in this procedure must be performed before results can be reported.

21. Quality issues are to be recorded on the QA sheet and in the quality logbook. Items of interest include but are not limited to maintenance performed anomalous control values, and instrument difficulties.

Note: Process changes and new method development must be performed under the direction and approval of toxicology supervision (i.e. the supervisor, work lead, or QA officer). A prove-in plan for process changes must be written and approved by supervision. Results of the prove-in activities must be documented and approved by supervision. See the Method Validation Procedure.

POLLUTION PREVENTION

1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in a laboratory operation. The United States Environmental Protection Agency (US EPA) has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of

first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot feasibly be reduced at the source, the US EPA recommends recycling as the next best option.

2. The quantity of chemicals purchased should be based on expected usage during the shelf life and disposal cost of unused material. Actual reagent preparation volume should reflect anticipated usage and reagent stability.

3. For information about pollution prevention that may be applicable to laboratories, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's (ACS) Department of Governmental Regulations and Science Policy, 1155 16th Street NW, Washington D.C. 20036, (202) 872-4477.

WASTE MANAGEMENT

1. The US EPA requires that laboratory waste management practice be consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from The American Chemical Society at the address listed above.

2. Dispose of all blood samples and blood-contaminated waste by placing in an autoclave bag labeled as biohazard and take to the autoclave for disposal.

3. Contact the Chemical Hygiene Office for disposal recommendation of other chemicals or solutions.

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