ANALYSIS OF VOLATILES IN AQUEOUS AND BIOLOGICAL SPECIMENS BY HEADSPACE GAS CHROMATOGRAPHY

10.1 POLICY

This test method may be used to confirm the presence of ethanol, acetone, isopropanol and methanol in aqueous and biological samples. Reporting of results following the application of this method will be contingent upon a thorough review and acceptance of quality control data and the qualification of individual results under the criteria for acceptance.

Any adjustments or deviations from the procedures below must be approved by the State Toxicologist, a Manager, or a Supervisor, and appropriately documented in the batch file.

10.2 PURPOSE

The purpose of this technical manual is to describe the identification and quantitation of ethanol, acetone, isopropanol (2-propanol) and methanol in aqueous and biological samples by headspace gas chromatography using an alcohol analysis capillary column and a flame ionization detector. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, and criteria for acceptance of volatile compounds.

10.3 PRINCIPLE

There is a direct relationship between the concentration of a volatile substance (e.g. ethanol) dissolved in a liquid (e.g. blood) and the concentration of the volatile substance in the vapor above the solution (headspace) for a given temperature, based on Henry's Law.

An aqueous or biological specimen is measured into a vial and then diluted with a measured volume of internal standard (n-propanol, 1-propanol). The vial is then sealed with a septum-equipped airtight seal. After a short incubation period at 70°C, the vial is pressurized and a measured aliquot of the headspace is transferred to the gas chromatograph for analysis.

Ethanol is resolved from other volatiles, such as acetone, isopropanol and methanol. Identification is by comparison of retention times of observed analytes to those present in the calibrators. Quantitation is accomplished by multilevel calibration. Each calibration level corresponds to a calibration sample with a known concentration of components. Confirmation is performed on a separate, identical instrument that is equipped with a separation column having different selectivity.

10.4 SPECIMENS

10.4.1 The specimen volume is 0.2 mL.

10.4.2 Specimens include whole blood, serum, plasma, urine, vitreous humor, tissue homogenate and aqueous samples. [NOTE: For liquor control board sample testing, refer to the Policy on Testing and Reporting Results for Liquor Control Board Samples.]

10.4.3 Dilutions of specimens may be analyzed at the Forensic Scientist's discretion; however, this should be done in addition to testing the standard specimen volume, unless sample quantity dictates otherwise.

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10.4.4 Analysis of larger specimen volumes must be approved and documented.

10.5 EQUIPMENT AND MATERIALS

10.5.1 EQUIPMENT

10.5.1.1 Disposable transfer pipettes (glass or polyethylene)

10.5.1.2 Microlab 500 Autopipette, Hamilton Automatic Diluter, or equivalent

10.5.1.3 Headspace autosampler vials (10 mL) and crimp tops

10.5.1.4 Cap crimper

10.5.1.5 Agilent (Hewlett Packard) 7694/G1888 headspace autosampler, or equivalent

10.5.1.6 Agilent (Hewlett Packard) 6890 gas chromatograph equipped with either a J&W DBALC1 capillary column (30 m x 0.53 mm ID x 3 µm film thickness) or a J&W DBALC2 capillary column (30 m x 0.53 mm ID x 2 µm film thickness), or equivalent

10.5.1.7 Computer system equipped with Agilent (Hewlett-Packard) ChemStation software

10.5.2 MATERIALS

10.5.2.1 Deionized water, laboratory grade (DI H_2O)

10.6 STANDARDS, CALIBRATORS AND CONTROLS

10.6.1 STANDARDS

10.6.1.1 Internal standard (n-propanol) is prepared and verified according to the Procedure for the Verification of n-Propanol Internal Standard.

10.6.2 CALIBRATORS

10.6.2.1 Ethanol calibrators are prepared and verified according to the Procedure for the Verification of Ethanol Calibrators.

10.6.2.2 Mixed volatile calibrators are prepared and verified according to the Procedure for the Verification of Mixed Volatile Calibrators and Control.

10.6.3 CONTROLS

10.6.3.1 Commercially prepared ethanol controls (CTRL) are purchased for use with each assay. The source and lot number of each control is documented in the Alcohol Control Log. The controls are verified according to the instructions on the Combined Ethanol Verification Worksheet. Verification is required prior to use. Controls are stored per manufacturer specifications.

a. Three ethanol-only controls are used, at the following concentrations:

CTRL 1	0.04 CTRL	0.04 g/100 mL

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CTRL 2	0.10 CTRL	0.10 g/100 mL
CTRL 3	0.20 CTRL	0.20 g/100 mL

b. Controls other than the aforementioned may be approved for use by the State Toxicologist or QA Manager, with appropriate documentation.

c. Ethanol controls are verified and considered approved for use when quantifying within the following inclusive ranges:

CTRL 1	0.038 – 0.042 g/100 mL
CTRL 2	0.095 – 0.105 g/100 mL
CTRL 3	0.190 – 0.210 g/100 mL

[NOTE: These ranges apply for initial verification of controls only.]

10.6.3.2 A positive mixed volatile control is prepared and verified according to the Procedure for the Verification of Mixed Volatile Calibrators and Control. The control is verified according to the instructions on the Combined Mixed Volatile Verification Worksheet. Verification is required prior to use.

a. One positive mixed volatile control is used, at the following concentration:

CTRL 4	60 CTRL	60 mg/dL

b. Controls other than the aforementioned may be approved for use by the State Toxicologist or QA Manager, with appropriate documentation.

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10.7 SAMPLE PREPARATION

10.7.1 Label 10 mL headspace vials for each member of the test batch (blank, negative controls, calibrators, positive controls, specimen samples, etc.) The batch should be set up according to the following sequence, where applicable:

1. Blank (DI H_2O, no ISTD)	10. CTRL 3 (0.20 g/100 mL)
2. CAL 1 (0.079 g/100 mL)	11. CTRL 4 (60 mg/dL) Volatile Control
3. CAL 2 (0.158 g/100 mL)	12. Negative Control
4. CAL 3 (0.316 g/100 mL)	13. Specimen #1
5. Negative Control (DI H_2O, plus ISTD)	14. Specimen #2
6. VOL 1 (40 mg/dL)	15. Specimen #3
7. VOL 2 (79 mg/dL)	16. (etc.)
8. CTRL 1 (0.04 g/100 mL)	Insert a positive & negative control after every 10 samples
9. CTRL 2 (0.10 g/100 mL)	Insert a positive & negative control at the end of the sequence

[NOTE: It is advisable to run an extra blank following any badly decomposed specimen or where volatiles other than ethanol are expected (e.g. toluene, difluoroethane).]

10.7.2 Equilibrate specimens to room temperature and mix before opening under a biohazard hood. Blood specimens are inspected to ensure the blood is mobile. If necessary, the sample may be sonicated or homogenized.

10.7.3 Aliquot 2.2 mL DI H_2O into the vial labeled blank and seal the vial tightly.

10.7.4 Using the auto-pipetter, aliquot 200 µL of the calibrators, controls or specimens and 2 mL of the internal standard solution into the respectively labeled headspace vial.

10.7.5 Seal the vial tightly.

10.7.6 Between each aliquot, rinse and wash the pipette tip appropriately (e.g. rinse pipette tip with diluted bleach and/or DI H_2O. Repeat if necessary.)

10.8 INSTRUMENTAL PARAMETERS

10.8.1 Load and edit a sequence on the headspace gas chromatograph. Enter the blank, calibrators, controls and specimens into the sequence table, and identify them appropriately under Sample Type.

10.8.2 Place each headspace vial in its respective position on the headspace autosampler and verify this placement against the sequence log.

10.8.3 Run the sequence under method BLDALCO. [Note: The method name may contain a numeric suffix to differentiate between instruments; for example BLDALCO1 for headspace instrument 1. A copy of the acquisition method for each headspace instrument is available at the instrument.]

10.8.4 If the batch size is larger than the capacity of the headspace autosampler, the analyst may run the remaining specimens and controls on the same instrument after the initial sequence has completed.

The initial sequence must end with a positive and negative control. The appended sequence must begin and end with both a positive and negative control. Any appended sequence must be injected on the same day as the initial sequence and aliquoted at the same time as the entire batch.

10.9 DATA ANALYSIS

10.9.1 Analysis of the batch data is conducted using the instrumental data analysis software in ChemStation. Mixed volatile data is acquired using the BLDALCO method and processed using the mixed volatile method VOL.

10.9.2 Quantitative calculations are generated by internal standard, multi-point, linear regression with equal weighting and the origin included.

10.9.3 The assigned value for each ethanol calibrator level in the batch calibration table matches those for the listed lot numbers as verified against a NIST curve.

10.9.4 Printed reports for each vial in the batch are generated for review, along with a copy of the calibration table applied to the batch.

10.9.5 Technical review of the batch is conducted according to the criteria listed below.

10.10 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

10.10.1 Blank

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10.10.1.1 The blank shall be devoid of any significant peaks[1].

10.10.2 Calibrators and calibration curves

10.10.2.1 Ethanol

a. Chromatographic peaks for ethanol and n-propanol shall appear symmetrical (i.e. no co-elution, split peaks, or shoulders).

b. Retention times for ethanol and n-propanol shall be within ±2% of those in calibrator 3. These are inclusive ranges.

c. Quantitative results for ethanol in each calibrator shall be within ±10% of their nominal values. These are inclusive ranges.

d. The calibration curve for ethanol shall have a correlation coefficient ≥0.99.

10.10.2.2 Mixed Volatile

a. Chromatographic peaks for acetone, isopropanol, methanol and n-propanol shall appear symmetrical (i.e. no co-elution, split peaks, or shoulders).

b. Retention times for acetone, isopropanol, methanol and n-propanol shall be within ±2% of those in mixed volatile calibrator 2. These are inclusive ranges.

c. Quantitative results for acetone, isopropanol and methanol in each mixed volatile calibrator shall be within ±10% of their nominal values. These are inclusive ranges.

d. The calibration curves for acetone, isopropanol and methanol shall have correlation coefficients ≥0.99.

10.10.3 Controls

10.10.3.1 The negative control(s) shall not identify ethanol above 0.005 g/100 mL. Identification is based on acceptable retention time matching and an integrated, symmetrical peak. All negative controls must meet these criteria for the batch to be accepted.

10.10.3.2 Positive ethanol controls

a. Chromatographic peaks for ethanol and n-propanol shall appear symmetrical.

b. Retention times for ethanol and n-propanol shall be within ±2% of those in calibrator 3. These are inclusive ranges.

c. Quantitative results for ethanol in each control shall be within ±10% of their nominal values. These are inclusive ranges.

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[1] Peaks appearing in the blank, calibrators, or positive and negative controls that are fully resolved from any volatile compound or internal standard are considered extraneous and not significant.

d. All positive ethanol controls must meet these criteria for the batch to be accepted.

10.10.3.3 Positive mixed volatile control

a. Chromatographic peaks for acetone, isopropanol, methanol and n-propanol shall appear symmetrical.

b. Retention times for acetone, isopropanol, methanol and n-propanol shall be within ±2% of those in mixed volatile calibrator 2. These are inclusive ranges.

c. Quantitative results for acetone, isopropanol and methanol in the control shall be within ±10% of their nominal values. These are inclusive ranges.

d. The positive mixed volatile control must meet these criteria for the batch to be acceptable for reporting of acetone, isopropanol and methanol. If the positive mixed volatile control does not meet these criteria, the acceptability of the batch for ethanol reporting is not affected, provided the positive ethanol controls meet criteria in 10.10.3.2.

10.11 CRITERIA FOR CASE SAMPLE ACCEPTANCE

If the criteria for batch acceptance have been satisfied, the results of individual case samples are deemed suitable for reporting if the following criteria are met.

10.11.1 Any chromatographic peak for ethanol, acetone, isopropanol or methanol shall appear symmetrical.

10.11.2 The retention time for ethanol and n-propanol are ±2% of those in calibrator 3. The retention times for acetone, isopropanol and methanol are ±2% of those in mixed volatile calibrator 2. These are inclusive ranges.

10.11.3 Quantitative results for ethanol are reported from 0.01 - 0.40 g/100 mL for living subjects and from 0.02 - 0.40 g/100 mL for postmortem samples. Quantitative results for acetone, isopropanol and methanol are reported from 10 - 79 mg/dL. These are inclusive ranges.

10.11.4 When dilutions of case samples are tested, the quantitative result(s) before multiplication shall be within the reporting range of the test method.

10.12 REPORTING

10.12.1 Blood ethanol results are reported according to the procedure found in the Policy on Reporting of Blood Alcohol Results.

10.12.2 For acetone, isopropanol and methanol, duplicate results must be within ±10% of the mean of the two results. The truncated, whole number, mean result is reported in units of mg/dL.

10.12.3 Liquor control board sample results are reported according to the Policy on Testing and Reporting Results for Liquor Control Board Samples.

10.13 DOCUMENTATION AND REVIEW

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10.13.1 In the event that a sequence is started on one day and completes after midnight, the date the sequence began will be the date of testing. Analysts will place their chromatograms, sequence tables and calibration tables and curves in a batch file.

10.13.2 The batch file will be forwarded to a reviewer for both a technical and administrative review. The reviewer will verify that the batch file contains all chromatograms, sequence tables and calibration tables and curves, all dates are correctly documented, the calibrator and control expiration dates have not been exceeded, individual chromatograms are initialed, all pages of the record are labeled with the batch number, and the calibrator and control values are within acceptable ranges. The reviewer will also verify that the batch meets the criteria for batch acceptance in 10.10 above.

10.13.3 The reviewer will sign and date the batch, indicating that the batch file is complete and the above procedures have been reviewed.

10.13.4 Upon completion of the technical and administrative review, the batch file is returned to the analyst.

10.13.5 The final batch file shall contain the calibration table and curves and all relevant sequence tables and chromatograms. Case sample chromatograms are filed in their respective case files.

10.14 REFERENCES

1. Agilent (Hewlett Packard) 7694 Headspace Autosampler Operating and Service Manual.
2. Agilent (Hewlett Packard) 6890 Gas Chromatograph manual (Operating manual 1 and 2).
3. *Medicolegal Aspects of Alcohol*, fifth edition. Garriott JC, ed. 2008. Lawyers & Judges Publishing Company, Inc., Tucson, AZ.
4. *Disposition of Toxic Drugs and Chemicals in Man*, eighth edition. Baselt RC, ed. 2008. Biomedical Publications. Foster City, CA.

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LIST OF CHANGES

Revision Date	Description	Page Number
08/20/12	Method approved by Washington State Toxicologist. See DRA dated 08/16/12. Method released for use in evidentiary testing on 08/20/12	All

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