

ETHANOL IN BIOLOGICAL SPECIMENS BY DUAL HEADSPACE GAS CHROMATOGRAPHY (HS/GC)

PRINCIPLE

Volatile compounds are analyzed in biological fluids by gas chromatography using N-propyl alcohol as the internal standard. Water containing an internal standard is added simultaneously with the biological sample as it is sampled using an automatic diluter. It is then sealed in a headspace sample vial prior to analysis. Volatile sample components are extracted from the non-volatile sample components by heating, pressurizing the vial and then sampling from the equilibrated gas phase above the sample phase. One milliliter of this gas phase mixture is injected onto a column, which splits into 2 different gas chromatographic columns. The volatile compounds are separated based on their respective molecular weights and polarities and detected with a flame ionization detector. The identification of ethanol and other volatile compounds is made by comparing the relative retention times of the unknown to the retention time of an internal standard. The ratio of sample peak area to internal standard peak area is compared to the calibration curve to provide a quantitation of volatile compounds in the sample.

By using 2 different columns that cause the volatiles to separate in different but known ways, a more specific identification is possible. The possibility of an interfering or co-eluting peak is also considerably reduced since it is unlikely to elute on both columns at the same retention time.

Samples are screened using the Volatiles Screen analytical method on the HS/GC. Positive samples are then quantified using the Ethanol Quantitation analytical method on the HS/GC.

SPECIMEN

1. **BLOOD:** Use blood specimens collected in gray top evacuated tubes (containing 100 mg sodium fluoride and 20 mg potassium oxalate). Blood specimens collected in other containers may be analyzed. The optimum sample size is 2 mL or greater. Specimens containing less than 2 mL may be analyzed.
2. **URINE:** Use urine specimens collected in urine collection bottles. Urine specimens collected in other containers may be analyzed. The optimum specimen size is 2 mL or greater. Specimens containing less than 2 mL may be analyzed.
3. **VITREOUS FLUID:** Use vitreous fluid specimens collected in red top tubes. Vitreous fluid specimens collected in other containers may be analyzed. The optimum specimen size is 2 mL or greater. Specimens containing less than 2 mL may be analyzed.
4. Keep specimens at 0°C to 8°C until analyzed; bring the specimens to room temperature before analysis.

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STANDARDS PREPARATION

Label all reagents with reagent name, tracking number, preparation date, preparer's initials, and expiration date. Store in a ground glass stoppered reagent bottle. Record the standard reagent information in LIMS.

1. Ethanol Working Standard Preparation:

After opening the ethanol standard ampoule, transfer the solution to its individual amber glass vial and cap with a screw cap. Standards are good for 2 weeks from the time they are opened. Store vials in refrigerator at 0°C to 8°C when not in use.

For the 0.79 g/dL Working Standard: Allow 200 proof ethanol to reach room temperature. Add 1 mL of ethanol to 100 mL de-ionized water and mix well. Transfer solution to an glass container and cap with a screw cap. This standard is good for 6 months from the date of preparation. Aliquots are good for 2 weeks from the time they are transferred. Store in refrigerator at 0°C to 8°C when not in use.

2. N-propyl Alcohol Internal Standard (IS) Preparation:

Add 200 µL N-propyl Alcohol to 2 L ultrapure water and mix well. Add 250 mg of sodium fluoride to prevent mold growth. Label reagent bottle with internal standard name, lot number, analyst's initials, and tracking number. Internal standard solution is good for 1 year from the date of preparation and can be stored at room temperature.

CONTROL PREPARATION

All controls must be prepared from a separate stock source than the standards. Separate vendors and/or lot numbers are sufficient to fill this requirement.

1. Whole Blood Reference Control:

Prepared by manufacturer. Store dried material at -10°C to -20°C. After reconstitution, store in the refrigerator at 0°C to 8°C; expiration is 30 days from the time it is opened.

2. Serum Interference Control (contains ethanol, methanol, acetone, isopropanol):

Prepare according to manufacturer instructions. Store in the refrigerator at 0°C to 8°C; expiration is 30 days from the time it is prepared.

CALIBRATION PROCEDURE

1. Calibrators consist of six levels: 0.01, 0.04, 0.08, 0.20, 0.40, 0.79 g/dL.

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Serum Reference Control

Whole Blood Control

≤ 20 screen injections

Check standard

≤ 20 screen injections

Check standard, etc.

7. Load the vials onto the autosampler tray according to the analytical sequence and analyze the samples with the Chemstation™ Ethanol program. Typical HS/GC conditions are included in the Instrument Log binder.
8. After the batch has been authorized by a technical review, prepare new aliquots of the whole blood control and the samples positive for ethanol as described in steps 1-5. Samples positive for methanol, acetone, and isopropanol will be quantitated according to the Volatiles Quantitation SOP.
9. The sequence order for quantitations continues on the batch in the following order:
 - Whole Blood Control
 - Check standard
 - ≤ 20 quantitation injections
 - Check standard
 - ≤ 20 quantitation injections
 - Check standard, etc.

CALCULATIONS

1. Calibrators: A calibration curve is derived by comparing the ratios of the calibrator ethanol peak areas to their respective internal standard peak areas. The ratio of sample peak area to internal standard peak area is compared to the calibration curve to provide a quantitation of any volatile compounds in the sample. The Chemstation™ software calculates a “least squares” line.
 - 1.1. The calibrator coefficient (r^2) must be ≥ 0.99 .
 - 1.2. A minimum of 3 calibrators is needed to construct the calibration curve.

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REPORTING RESULTS

1. Calibration results are printed on hardcopy.
2. Blank control and Serum control results are printed and entered as "PASS" or "FAIL" on LIMS. No additional blank or serum controls are included for quantitation.
3. Whole Blood Control and Check Standard results:
 - 3.1. For screens: results are printed and entered as "PASS" or "FAIL" on LIMS.
 - 3.2. For quantitations: results are printed and entered to the third decimal place on LIMS.
4. Sample results:
 - 4.1. For screens: results are printed and entered as "Positive" or "Negative" on LIMS.
 - 4.2. For quantitations: results are printed and entered to the third decimal place on LIMS.
5. Reagent information and batch QC information are entered on LIMS. Notes relating to batch QC information are included under "Criteria Exceptions". Other notes relating to the batch are included under "Batch Comment."

LIMITATIONS OF PROCEDURE

1. For Ethanol:
 - 1.1. The LE reporting limit = 0.01 g/dL.
 - 1.2. The ME reporting limit = 0.02 g/dL.
 - 1.3. The upper reporting limits for both LE and ME cases = 0.79 g/dL.
2. For Methanol, Acetone, and Isopropanol: the screen lower reporting limits are:

Analyte	Headspace 1 Response	Headspace 2 Response
Methanol	10	5
Acetone	50	15
Isopropanol	100	30

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APPENDIX A: Relative Retention Times (RRTs) for Miscellaneous Volatile Compounds

The following table provides a summary of RRTs of other volatile compounds on the two different columns of the HS/GC. With only one exception (chloroform), all tested interferences caused conflicts in only 1 column and may be compensated for by using data from the other column.

The minor peaks for chloroform are the only known interferences for both columns, and this occurs only when the chloroform has broken down. When fresh chloroform is present, no minor peaks were observed. If the main chloroform peak is present then it *may* contribute to any ethanol seen.

Elution Order DB-Alc1			Elution Order DB-Alc2		
#	Compound	RRT db-alc1	#	Compound	RRT db-alc2
1	Methanol	0.493	1	Acetaldehyde	0.435
2	Formaldehyde	0.501/0.833	2	Methanol	0.464
3	Acetaldehyde	0.542	3	Formaldehyde	0.474 / 0.580
4	Ethanol	0.616	4	Ethyl ether	0.516
5	Ethyl ether	0.736	5	Ethanol	0.584
6	Isopropyl alcohol	0.746	6	Acetone	0.642
7	Methylene chloride	0.826	7	Hexane	0.666 /m
8	Acetone	0.898	8	Isopropyl alcohol	0.683
9	Acetonitrile	0.907	9	Methylene chloride	0.697
10	N-propyl alcohol (IS)	1.000	10	Acetonitrile	0.794
11	Hexane	1.146 /m	11	N-propyl alcohol (IS)	1.00
12	Chloroform	1.285 /m	12	Ethyl acetate	1.05 /m

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AUTHORIZATION

QA Manager Approval: Signature: *Belesang* Date: 3/14/12
Bureau Director Approval: Signature: *[Signature]* Date: 3.13.12
Laboratory Director Approval: Signature: *[Signature]* Date: 3/15/12
Division Director Approval: Signature: *[Signature]* Date: 3/15/12