ANALYSIS OF ALCOHOLS IN AQUEOUS AND BIOLOGICAL SAMPLES BY HEADSPACE GAS CHROMATOGRAPHY

A. *Introduction:*

Headspace Gas Chromatography is a useful and accurate method to analyze ethanol and other volatile substances in blood and other tissues. Use of two complimentary systems precludes co-elution of other volatiles interfering with ethanol quantification. Results are reported in units of grams of alcohol per 100mL of whole blood, as required by the Washington Administrative Code (WAC 448.14)

B. *Principle and Purpose*

There is a direct relationship between the concentration of a volatile substance (such as ethanol) dissolved in a liquid (such as blood) and the concentration of the volatile substance in the vapor above the solution for a given temperature based on Henry's Law. Headspace gas chromatography utilizes this principle to accurately quantify ethanol and other volatiles in biological fluids and tissues. The volatility of ethanol relative to the aqueous biological specimen is used to separate the volatile from the matrix. The solution is placed in an airtight container and the amount of volatile in the air space above the liquid is proportional to the concentration of the volatile liquid in the solution. Therefore, sampling the headspace of heated specimens and similarly treated ethanol calibrators allows calculation of the ethanol concentration in the specimen.

The headspace vapor is injected onto a capillary column. Separation of different volatiles takes place in the column according to the size of the analytes. A flame ionization detector (FID) is used; wherein a hydrogen/air flame burns at the jet tip and the column effluent exits through the jet into the flame. A constant electrical potential is maintained between the jet and the collector and the gap acts as a variable resistance. When just gas is flowing, this is monitored as baseline. As analyte molecules are ionized in the flame, the resistance decreases, more current flows and this amplified current is the detector response.

The unknown samples are diluted with a solution containing n-propanol as the internal standard and sodium chloride to increase the partial pressure of ethanol and n-propanol.

C. *Acceptable sample types and volumes:*

Serum, plasma, whole blood, vitreous humor, tissue homogenates, urine and aqueous solutions are appropriate samples for analysis. (Solid tissues are weighed, homogenized in deionized water and reported in gm/kg.) The volume of sample is 0.2 mL and is diluted with 2.0 mL diluent. Samples with high concentrations of volatiles may be further diluted with water for re-analysis to get the result within the limits of linearity for the assay. Alternatively, a 1:2 dilution can be using the diluter to dispense 0.1 mL of sample with 2.0 mL of diluent.

Approved: Date: 10/23/04

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D. Calibration Standards:

The headspace gas chromatograph is calibrated for each "batch". The calibration must be within 24 hours of the time the sample is analyzed for the results to acceptable. For analyses other than a complete batch (repeat samples, certification runs, etc) a previous calibration may be used, if there has been no change to the internal standard, at least one contemporary control is analyzed and the calibration is within 24 hours of the last sample analyzed. The accuracy of the calibration is verified against external quality control samples. See below. The standards are prepared once each week, at a minimum. Solution proportions are calculated based on ethanol density of 0.79gm/mL.

For the purposes of this SOP, a batch is defined as all samples run by one analyst, within a 24 hour period, using the same internal standard, with the same calibration, even if there is more than one sequence.

Materials

Absolute ethanol, used within 6 months of the date it is first opened.
Water (deionized, or distilled)
1mL volumetric pipette, grade A
Volumetric flasks (250, 500, 1000mL), grade A
plastic storage bottles

Using the grade A volumetric glassware, prepare the following:

Standard Concentration	Preparation
Blank	water only
0.079 gm/100 mL	1 mL of ethanol in 1000 mL H_2O
0.158 gm/100 mL	1 mL of ethanol in 500 mL H_2O
0.316 gm,/100 mL	1 mL of ethanol in 250 mL H_2O

Preparation of the Ethanol Calibration Standards is documented in the Alcohol Standard Log (see Appendix A).

Standards are labeled, tightly sealed and refrigerated at 5 degrees C. when not in use. They are brought to room temperature before use.

E. *Internal Standard:*

The internal standard is prepared as follows:

20 gm sodium chloride
0.3 mL n-propanol
diluted to 2 L water.

Mix thoroughly and store at room temperature in a sealed container.

Internal standard is stored at room temperature. Preparation of internal standard is documented in the Alcohol Standard Log. Internal Standard expires 30 days after preparation.

F. *Controls:*

Commercially prepared controls are purchased for use in each assay. At a minimum, two (2) control levels are included in each batch. Reanalysis of some samples, a certification run or other limited run must include at least one control per 10 samples. See Appendix B for a list of current controls. After every 10 unknowns, one quality control sample followed by one blank is analyzed.

G. *Non-calibration Standards:*

A 0.02 gm/100 mL standard is analyzed with each assay.

0.02 gm/100 mL

1 mL absolute ethanol in 4 L H_2O

0.02 Standard is tightly sealed, labeled and refrigerated at 5 degrees C. It is brought to room temperature before use.

Preparation of 0.02 gm/100mL standard is documented in the Alcohol Standard Log and expires 90 days after preparation. 1102-04 AM

Volatile standards – Two concentrations of commonly encountered volatiles are included as volatile standards in each assay. The two volatiles standards are prepared as follows:

11-2-04 AM

0.04 gm/100 mL Volatiles	0.079 gm/100mL Volatile standard 11-2-04 AM
1 mL ethanol	1 mL ethanol
1 mL acetone	1 mL acetone
1 mL ispropanol	1 mL ispropanol
1 mL methanol	1 mL methanol
in 2 L H_2O	in 1 L H_2O

Preparation of the volatile Standards is documented in the Alcohol Standard Log (see Appendix A).

Volatile standards are tightly sealed, labeled and refrigerated at 5 degrees C. They are brought to room temperature before use.

Volatile standards expire 90 days after preparation.

H. *Equipment:*

Agilent (Hewlett Packard) Network Headspace Autosampler or equivalent
Agilent (Hewlett Packard) 6890 or 6890N gas chromatograph; equipped with a J&W DBALC1 megabore (0.53 mm) 30 meter capillary column and/or a J&W DBALS2

megabore (0.53 mm) 30 meter capillary column or equivalent. (For information on the columns, see Appendix C)
Computer System equipped with HP GC Chem Station
Compressed gases; air, nitrogen , hydrogen, helium
Autosampler vials
Cap Crimper
Hamilton Automatic Diluter

I. *Sample Handling:*

All biological samples must be treated as potentially infectious. The blood tube (vacutainer) is initially opened inside of a biological or chemical hood with the fan hood on, to protect the analyst from potential aerosol hazard. Precautions should be used to limit exposure to blood and aerosols. The blood sample is inspected, to ensure that the blood is mobile. If the blood appears to be clotted, it may be necessary to homogenize the blood in a tissue homogenizer, prior to aliquoting. *NOTE: ALL TISSUE AND BLOOD HOMOGENIZATION MUST BE CONDUCTED INSIDE A BIOLOGICAL OR CHEMICAL HOOD.*

J. *Analysis:*

1) All unknown samples, standards and controls are analyzed in duplicate.

2) The following standards are used as calibrators:
0.079, 0.158, 0.316 g/100mL
Following the high standard, a blank is analyzed to verify the absence of carryover.

3) A minimum of two control samples is analyzed following the calibrators (and prior to the unknowns) to verify the calibration.

4) The following standards are included in the analysis:
0.02 gm/100 mL
0.04 Volatile Mix
0.079 Volatile Mix

5) Auto-pipette 200 µL of blood, control, or standard solution into a 10 mL autosampler vial. Add 2 mL of internal standard solution. Seal the vial tightly and shake well until homogeneous. Note: If other than 200 µL is aliquotted, this must be noted on in the sample information in the sample log table.

6) Alternating controls (different levels) are repeated periodically throughout the run followed by a blank. Each positive sample should be separated from a commercial control and a blank by no more than ten other samples. If all the samples are not analyzed at once, the first aliquot should be a control, followed by a blank.

7) Samples are analyzed in duplicate, once on each of two headspace systems, unless otherwise approved by the laboratory manager and or the State Toxicologist due to equipment limitations. (Under certain circumstances, the duplicates may be analyzed

on the same instrument, using two different runs and two different calibrations. This is documented in a memorandum for record.

8) Prepare the sample worklist of unknown samples, standards and controls. (Note: it is advisable to run an extra blank following any badly decomposed sample.) One or more headspace instruments may be controlled by a single computer. On the toolbar in Method and Run Control, select Sequence - Sequence Parameters. Identify the operator and establish a unique subdirectory for the data. The subdirectory should identify the date the analysis was started. By convention, the subdirectory is numerical date (YYMMDD) followed by initial(s) of the analyst, if more than one subdirectory is run on a given day, a modifier is appended to the analyst's initials.

9) Select Sequence, Sequence Table. Enter the standards controls and unknown samples into the sequence table. The calibrators, 0.079 gm/100 mL, 0.158 gm/100 mL and 0.316 gm/100 mL are identified in sample type as calibrators 1, 2 and 3, respectively. The blank following the high standard is identified as a Ctrl Samp in Sample Type as are all of the controls throughout the run. Each unknown and the other standards and blanks are identified as SAMP in the sample type. A maximum of 44 or 70 samples may be included in one run dependent upon the headspace model.

10) Identify the method as BLDALCO (a modifier may identify the instrument). The injection number is 1. Print the sequence table for each instrument. (The method for the two volatile standards may be selected as VOLATILE.)

11) Open the top of the headspace autosampler. Place the autosampler vials in the numbered positions according to the positions identified in the sequence log table.

12) Select the HP6890 System monitor and click on the Vials icon to specify the number of vials in the run if analyzing on 6890. (This is not required on 6890N.) Click on the start icon. Repeat for the other instrument.

13) Return to the Sequence Table for in Instrument Method & Run Control and select "Run Sequence" for each instrument. Note that the headspace and the software must be started independently on some models.

14) The GC methods for each instrument are found in Appendix D.

15) At the conclusion of the run, it advisable to review the data before removing the vials from the autosampler. Autosampler vials are discarded in biohazardous waste.

16) A sample chromatogram is found in Appendix E.

K. *QUALITY CONTROL AND DATA REVIEW:*

1) Ensure that the blank following the high standard does not have any peaks present. Ensure that all blanks following control samples are devoid of peaks. (Blanks following decomposed samples may have peaks.)

Approved: _____ Date: 10/27/04

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2) Verify the presence of the internal standard in each analysis. To ensure sensitivity the ISTD area must be at least 1000. Low ISTD area counts may be indicative of a clogged injector needle.

3) Verify that each control is properly identified and quantifies within ± 0.01 gm/100 mL of the target value. Verify that the other standards quantify within ± 0.01 gm/100 mL of their respective target value.

   i. If any quality control values are out of range or any of the blanks following a quality control are positive, determine if it is due to incorrect placement of the sample vials in the autosampler. This can be corrected only before any vials are removed from the autosampler, as follows:
      a) Compare the written numbers on the autosampler vials in each position with the sample ID on the sequence log table.
      b) If there is a mismatch, the samples can be moved to the correct position and a partial sequence analyzed.

   ii. If one quality control per analytical run is out of range but within +/- 0.02 gm/100 mL of the target value, all positive samples within 10 samples of the failed QC are realiquotted and reanalyzed.
      a) Use the same calibration, if it is within 24 hours of the original calibration time.
      b) Use the same internal standard.
      c) Include a positive control and a blank at the beginning and end of the partial sequence.

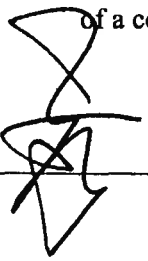
   iii. If more than one QC is out of range or the partial sequence in "i" does not resolve the problems, the entire run is realiquotted, including standards and controls and rerun.

4) For any Out-of-Range Quality Control, clearly document the QC failure and the corrective action taken on the QC Out of Range Log, Appendix F and indicates which samples were re-analyzed along with the failed controls. This is reviewed, monthly, by the technical lead.

5) For any data which was re-analyzed due to control failure or other reason, draw one line through the failed data with a brief comment as to the reason for failure, initial and date the annotation and maintain this in the corresponding case folder.

6) Verify that for each unknown sample, the duplicate results agree to within ± 0.01 (% BAC) gm/100 mL from the mean (inclusive). Report the average of the two values, rounding to two decimal places, using the mathematical rules of rounding. If the duplicate results are not within ± 0.01 gm/100 mL, the sample is rediluted and reassayed on two instruments. It may be necessary to homogenize the sample before reanalysis.

   i. Include one quality control sample and a blank at the beginning or end of the realiquotted sequence, insuring that all samples are within 10 samples of a control.

Approved: 

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a) If the original calibration is within 24 hours and there have been no changes to the internal standard, recalibration is not necessary.

b) If the original calibration is outside of 24 hours or there has been a change in the internal standard, recalibration is required.

7) Examine each unknown for the presence of other peaks in the chromatogram. If other peaks are identified, determine if they are one of the volatiles found in the volatile mix. If they are, they may be quantified in the offline method.

i. In the Offline Mode of Instrument Method & Run Control Panel, load the volatile method, in duplicate.
ii. Identify the file, which contains the 0.04 volatile standard, and load it. On the toolbar, select Calibration. Select recalibrate, select level 1 and click on replace. Identify and load the 0.079 volatile standard, and update as calibrator #2.
iii. Identify any unknown with volatiles and load the files. Select Generate Report. The printout will quantify acetone, methanol, isopropanol (as well as ethanol). See appendix G for a printout of the 2 calibrators and an unknown recalculated with the volatile method.

8) If the extraneous peaks are not acetaldehyde and are not identified as one of the other volatiles, run the sample (or the previous sample if there is an indication that it is a late peak from a previous sample) by Headspace GC, toluene method, in an attempt to identify the volatile. Refer to the interfering substance manual in the laboratory for assistance with identification of unknown peaks. Appendix H lists of some commonly identified volatiles and their relative retention times on each currently used instrument.

9) It may be necessary to heat the vial and inject the vapor for analysis by GCMS for identification. To quantify any volatile, appropriate standards must be concurrently analyzed with the sample and the contemporary standards filed in the folder with the sample data.

10) Samples with concentrations greater than 0.800g/100mL must be diluted appropriately and re-analyzed.

11) Place all chromatograms into the respective files. Initial standards and all controls (and subsequent blanks) are filed with the first sample of the run. Include all chromatograms in the file, even if the sample is reanalyzed. Line through unacceptable data, initial and date and add a brief explanation for the reanalysis.

12) If it is necessary to reprint a chromatogram, note that the sample is always recalculated when it is printed, based upon the most recent calibration curve. If the instrument has not been used since the sample was run, the sample may be reprinted. If it has, take the following action:

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i. Load the contemporary calibrators and update the calibration curve.
ii. Identify the files to be reprinted.
iii. Load each file and generate the report. Print the contemporary calibrators.
iv. DO NOT REPRINT A RESULT WITHOUT RELOADING THE CONTEMPORARY CALIBRATOR.
v. Include the reprinted calibrators with the reprinted data in the file or note in which file it can be located.

L. *Interpretation of results:*

1) Post mortem samples: Blood alcohol results of 0.019 g/100 mL or less shall be reported as negative.

2) Samples drawn from living subjects: Blood alcohol results of 0.009g% or less shall be reported as negative.

Ante-mortem samples collected prior to death are reported using the rules for living subjects.

3) The following clinical effects and symptoms are associated with various blood alcohol levels (Caplan, 1982).

BAC (g%)	***clinical effects and symptoms***
0-0.06	no apparent influence by ordinary observations; slight changes detectable by special tests
0.03-0.12	euphoria, sociability, decreased inhibitions, diminished attention, judgement and control, loss of efficiency in performance tests
0.09-0.25	emotional instability, loss of critical judgement, decreased sensitory response, impaired memory and comprehension, some muscular incoordination, decreased reaction time.
0.18-0.30	disorientation, mental confusion, dizziness, loss of emotional control, impaired balance, muscular incoordination, slurred speech decreased pain perception
0.27-0.40	apathy, inertia, marked decrease to stimuli and advanced muscular incoordination, vomiting, incontinence, sleep or stupor
0.35 and above	partial or complete unconsciousness, coma, respiratory distress, circulatory failure, possible death

The signs and symptoms reported at all levels may significantly impair driving regardless of their severity or detectability. Note that tolerance to alcohol such as that present in conditioned drinkers can cause these effects to be less obvious in some individuals.

Approved:

Barry K. Logan, PhD

Date: 10/23/04

M. *References:*

Y.H. Caplan in "Forensic Science Handbook vol. 1." R. Saferstein (ed.) Prentice Hall, 1982.

"Goodman and Gilman's the Pharmacological Basis of Therapeutics", McMillan publishing, 7th ed., 1985 .

Agilent (Hewlett Packard) 7694 Headspace Autosampler instruction manual

Agilent (Helwett Packard) 6890 Gas Chromatograph manual

James C. Garriott "Analysis for Alcohol in Postmortem Specimens" in Medicolegal Aspects of Alcohol J. Garriott (ed.) Lawyers and Judges Publishing Co. 3rd edition 1996.

STATEMENT OF STATE TOXICOLOGIST -

In my capacity as Washington State Toxicologist, and by my authority outlined in RCW 46.61.506, I have reviewed this protocol and find it to be proper and adequate in form and substance for the purpose it was intended. I therefore approve and authorize its use. This protocol replaces all previous headspace GC analysis protocols and ethanol standard preparation protocols. This supplements the simulator solution protocol dated 05/27/03, which remains in effect.

Barry K. Logan Ph.D.
Washington State Toxicologist — Date: 10/23/04

Reviewed By: Ann Marie Gordon
Laboratory Manager — Date: 10/29/04

The following toxicologists have read the Headspace GC Protocol and agree to follow the procedure as it is written. Any deviations from the procedure must be documented in writing and approved by the laboratory manager or the State Toxicologist.

Reviewed By: Dorota Schranz, PhD
Technical Lead — Date:

Reviewed By: Edward Formoso
Supervisor — Date: 11/2/04

Reviewed By: Melissa Pemberton
Supervisor — Date: 11/1/04

Approved: Barry K. Logan, PhD — Date: 10/23/04

Reviewed By: Kari Gruendell Date: 11/1/04

Reviewed By: W S Marshall Date: 11/2/04

Reviewed By: [signature] Date: 11/2/04

Reviewed By: [signature] Date: 11/02/04

Reviewed By: [signature] Date: 11-2-04

Reviewed By: [signature] Date: 11/3/04

Reviewed By: [signature] Date: 11/3/04

Reviewed By: [signature] Date: 11/5/04

Reviewed By: [signature] Date: 11/8/04

Reviewed By: [signature] Date: 11/23/04

Approved: [signature]

Barry K. Logan, PhD

Date: 10/23/04

Date	Standard	Analyst

Approved:

Barry K. Logan, PhD

Date: 8/5/04

Appendix B:

Current External Alcohol Controls

As of 10-23-02:

CAP - 0.04 gm ethanol/100 mL

Restek - 0.04 gm ethanol/100 mL

Restek – 0.10 gm ethanol/100 mL

Restek – 0.20 gm ethanol/100 mL

The certifications provided by the control manufacturer are filed in the Quality Control File.

Approved:

Barry K. Logan, PhD

Date: 8/5/04

Appendix C:

Column Information - 2 Pages

Approved:

Barry K. Logan, PhD

Date: 8/5/04

Method Information

Acetaldehyde

Method Change History

Operator	Date	Change Information
EGLE WEISS	4/9/02 8:47:10 AM	
WP MARSHAL	5/13/02 7:45:50 AM	
EGLE WEISS	5/24/02 9:24:32 AM	
WP MARSHAL	6/13/02 9:38:18 AM	
Estuardo J	6/23/02 11:29:14 AM	
WP MARSHAL	6/26/02 12:26:28 PM	
WP MARSHAL	6/26/02 12:28:44 PM	
WP MARSHAL	6/26/02 2:11:00 PM	
RUTH LUTHI	10/4/02 12:17:39 PM	
RUTH LUTHI	11/1/02 9:10:14 AM	
WP MARSHAL	11/14/02 12:04:23 PM	

Run Time Checklist

Pre-Run Cmd/Macro: off

Data Acquisition: on

Standard Data Analysis: on

Customized Data Analysis: on
Macro Name: macro "contres1.mac",go

Save GLP Data: off

Post-Run Cmd/Macro: off

Save Method with Data: on

Injection Source and Location

Injection Source: Manual

Injection Location: Front

HP6890 GC METHOD

OVEN
Initial temp: 40 'C (On)
Initial time: 2.20 min
Ramps:
Rate Final temp Final time
1 0.0(Off)
Post temp: 50 'C
Post time: 0.00 min
Run time: 2.20 min
Maximum temp: 225 'C
Equilibration time: 0.50 min

FRONT INLET (PURGED PACKED)
Initial temp: 250 'C (On)
Pressure: 9.53 psi (On)
Gas type: Nitrogen

BACK INLET (SPLIT/SPLITLESS)
Mode: Split
Initial temp: 50 'C (Off)
Pressure: 0.00 psi (Off)
Total flow: 45.0 mL/min
Gas saver: Off
Gas type: Nitrogen

COLUMN 1
Capillary Column
Model Number: J&W 125-9134
DB-ALC1
Max temperature: 280 'C
Nominal length: 30.0 m
Nominal diameter: 530.00 um
Nominal film thickness: 3.00 um
Mode: constant flow
Initial flow: 16.6 mL/min
Nominal init pressure: 9.53 psi
Average velocity: 100 cm/sec
Inlet: Front Inlet
Outlet: Front Detector
Outlet pressure: ambient

COLUMN 2
(not installed)

FRONT DETECTOR (FID)
Temperature: 250 'C (On)
Hydrogen flow: 40.0 mL/min (On)
Air flow: 300.0 mL/min (On)
Mode: Constant column+makeup flow
Combined flow: 40.0 mL/min
Makeup flow: On
Makeup Gas Type: Nitrogen
Flame: On
Electrometer: On
Lit offset: 2.0

BACK DETECTOR (NO DET)

SIGNAL 1
Data rate: 5 Hz
Type: front detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 10 Hz
Type: front detector
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
Derive from front detector

COLUMN COMP 2
Derive from front detector

AUX PRESSURE 3
Description:
Gas Type: Nitrogen
Initial pressure: 0.00 psi (Off)

AUX PRESSURE 4
Description:
Gas Type: Nitrogen
Initial pressure: 10.00 psi (On)
Initial time: 650.00 min
Rate Final pres Final time
1 0.0(Off)

AUX PRESSURE 5
Description:
Gas Type: Nitrogen
Initial pressure: 0.00 psi (Off)

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

Integration Events

Results will be produced with the enhanced integrator.

Default Integration Event Table "Event"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_TCD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ADC"

Event	Value	Time
Initial Slope Sensitivity	20.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ECD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_NPD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FPD"

Event	Value	Time
Initial Slope Sensitivity	50.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_uECD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FID"

Event	Value	Time
Initial Slope Sensitivity	50.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	50.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Apply Manual Integration Events: No

Calibration Table

Calib. Data Modified : Tuesday, May 27, 2003 1:41:00 PM

Calculate : Internal Standard
Based on : Peak Area

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.050 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.050 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Floating Average New 99%
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Default Sample ISTD Information (if not set in sample table):

ISTD #	ISTD Amount [ng/ul]	Name
5	1.00000	n-Propanol

Signal 1: FID1 A,

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref Grp	Name
1.041	1	1	7.90000e-2	1175.00891	6.72335e-5	5	Ethanol
		2	1.58000e-1	2330.87671	6.77857e-5		
		3	3.16000e-1	4638.43408	6.81264e-5		
1.699	1	1	1.00000	3314.00049	3.01750e-4	I5	n-Propanol
		2	1.00000	3299.82178	3.03047e-4		
		3	1.00000	3283.34692	3.04567e-4		

Peak Sum Table

No Entries in table

Method Information

Method Change History

Operator	Date	Change Information
EGLE WEISS	4/9/02 8:49:07 AM	
RUTH LUTHI	6/5/02 7:44:30 AM	
EGLE WEISS	6/25/02 10:26:52 AM	
RUTH LUTHI	7/19/02 3:23:55 PM	
Estuardo J	8/5/02 11:08:20 AM	
RUTH LUTHI	9/12/02 7:28:32 AM	
Jayne E. T	9/30/02 6:00:25 AM	
Jayne E. T	9/30/02 11:20:11 AM	
Jayne E. T	9/30/02 11:35:53 AM	
RUTH LUTHI	10/4/02 12:18:58 PM	
RUTH LUTHI	11/1/02 9:11:21 AM	
WP MARSHAL	11/14/02 12:04:39 PM	
ED FORMOSO	4/7/03 8:47:18 AM	

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	on
Macro Name:	macro "contres2.mac",go
Save GLP Data:	off
Post-Run Cmd/Macro:	off
Save Method with Data:	off

Injection Source and Location

Injection Source:	Manual
Injection Location:	Front

HP6890 GC METHOD

OVEN
- Initial temp: 37 'C (On)
- Initial time: 2.20 min
- Ramps:

#	Rate	Final temp	Final time
1	0.0(Off)		

- Post temp: 50 'C
- Post time: 0.00 min
- Run time: 2.20 min
- Maximum temp: 120 'C
- Equilibration time: 0.50 min

FRONT INLET (PURGED PACKED)
- Initial temp: 250 'C (On)
- Pressure: 10.02 psi (On)
- Gas type: Nitrogen

BACK INLET (SPLIT/SPLITLESS)
- Mode: Split
- Initial temp: 50 'C (Off)
- Pressure: 0.00 psi (Off)
- Total flow: 45.0 mL/min
- Gas saver: Off
- Gas type: Helium

COLUMN 1
- Capillary Column
- Model Number: J&W 125-9134
- DB-ALC1
- Max temperature: 280 'C
- Nominal length: 30.0 m
- Nominal diameter: 530.00 um
- Nominal film thickness: 3.00 um
- Mode: constant flow
- Initial flow: 18.0 mL/min
- Nominal init pressure: 10.03 psi
- Average velocity: 106 cm/sec
- Inlet: Front Inlet
- Outlet: Front Detector
- Outlet pressure: ambient

COLUMN 2
- (not installed)

FRONT DETECTOR (FID)
- Temperature: 250 'C (On)
- Hydrogen flow: 40.0 mL/min (On)
- Air flow: 300.0 mL/min (On)
- Mode: Constant column+makeup flow
- Combined flow: 40.0 mL/min
- Makeup flow: On
- Makeup Gas Type: Nitrogen
- Flame: On
- Electrometer: On
- Lit offset: 2.0

BACK DETECTOR (NO DET)

SIGNAL 1
- Data rate: 5 Hz
- Type: front detector
- Save Data: On
- Zero: 0.0 (Off)
- Range: 0
- Fast Peaks: Off
- Attenuation: 0

SIGNAL 2
- Data rate: 10 Hz
- Type: front detector
- Save Data: Off
- Zero: 0.0 (Off)
- Range: 0
- Fast Peaks: Off
- Attenuation: 0

COLUMN COMP 1
- Derive from front detector

COLUMN COMP 2
- Derive from front detector

AUX PRESSURE 3
- Description:
- Gas Type: Helium
- Initial pressure: 0.00 psi (Off)

AUX PRESSURE 4
- Description:
- Gas Type: Helium
- Initial pressure: 0.00 psi (Off)

AUX PRESSURE 5
- Description:
- Gas Type: Nitrogen

Initial pressure: 10.00 psi (On)
Initial time: 0.00 min

#	Rate	Final pres	Final time
1	0.0(off)		

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

Integration Events

Results will be produced with the enhanced integrator.

Default Integration Event Table "Event"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_TCD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ADC"

Event	Value	Time
Initial Slope Sensitivity	20.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ECD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_NPD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FPD"

Event	Value	Time
Initial Slope Sensitivity	50.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_uECD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FID"

Event	Value	Time
Initial Slope Sensitivity	75.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	75.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Apply Manual Integration Events: No

Calibration Table

Calib. Data Modified : Tuesday, May 27, 2003 1:41:40 PM

Calculate : Internal Standard
Based on : Peak Area

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.040 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.040 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Floating Average New 99%
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Default Sample ISTD Information (if not set in sample table):

ISTD #	ISTD Amount [ng/ul]	Name
1	1.00000	n-Propanol

ignal 1: FID1 A,

RetTime [min]	Sig	Lvl		Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
1.057	1	1	1	7.90000e-2	1092.32947	7.23225e-5	1		Ethanol
			2	1.58000e-1	2195.83130	7.19545e-5			
			3	3.16000e-1	4341.76953	7.27814e-5			
1.847	1	1	1	1.00000	2942.46924	3.39851e-4	I1		n-Propanol
			2	1.00000	2961.02295	3.37721e-4			
			3	1.00000	2934.91602	3.40725e-4			

Peak Sum Table

No Entries in table

J&W Column Performance Summary

Part No.: 1259134Z
Column I.D. No.: 9919723Z
Liquid Phase: DB-BAC-1
Film Thickness: 3.00 µm
Column Dimensions:
30 m x 0.542 mm
Temperature Limits:
20 °C to 260 °C (280 °C Program)

Theoretical Plates/Meter:
0

UTE%: (1)
NOT APPLICABLE

Retention Index:
NOT APPLICABLE

Peak Height Ratio:
NOT APPLICABLE

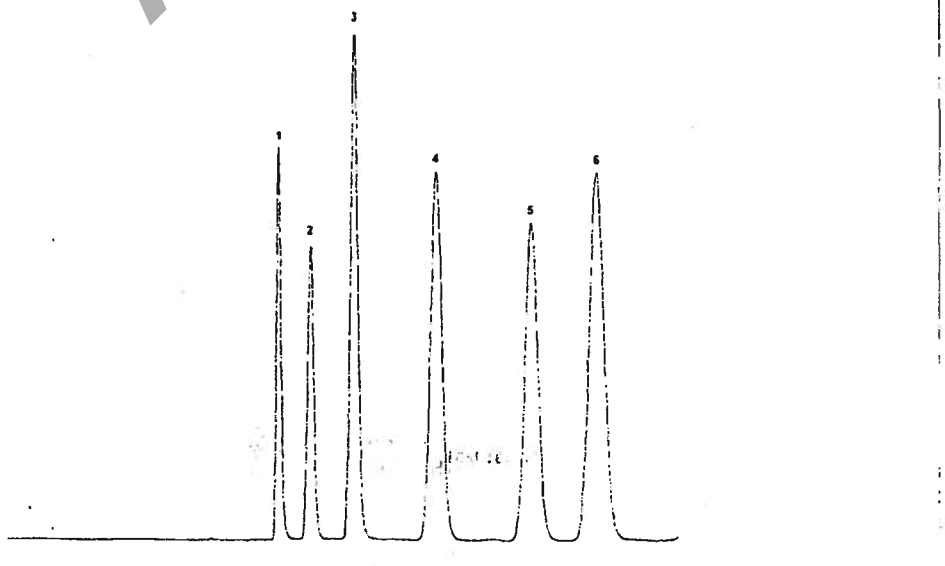
Resolution:
NOT APPLICABLE

Compound Identification	Retention Time (t_R)	Partition Ratio (k)	Peak Width ($W_{1/2}$)
1. METHANOL	0.89	0.0	0.016
2. ACETALDEHYDE	1.00	0.1	0.023
3. ETHANOL	1.15	0.3	0.028
4. ISOPROPANOL	1.43	0.6	0.042
5. ACETONE	1.74	1.0	0.047
6. 1-PROPANOL	1.96	1.2	0.054

(t_0)

Test Temperature: 40 °C
Carrier Gas: (He) 55.9 $\frac{cm}{sec}$ (7.7 $\frac{mL}{min}$)
Injection: Split
Analyst: MICHAEL

RECEIVED
NOV 2 9 1999



IMPORTANT: KEEP THIS DOCUMENT FOR YOUR RECORDS

6/14/2004 BKL
BKL 5/27/10

J&W Column Performance Summary

Part No.: *1259234Z*
Column I.D. No.: **9994526Z**
Liquid Phase: **DB-ALC2**
Film Thickness: 2.00 µm
Column Dimensions:
30 m x 0.530 mm
Temperature Limits:
20 °C to 260 °C (280 °C Program)

Theoretical Plates/Meter:

UTE%: (1)
NOT APPLICABLE

Retention Index:
NOT APPLICABLE

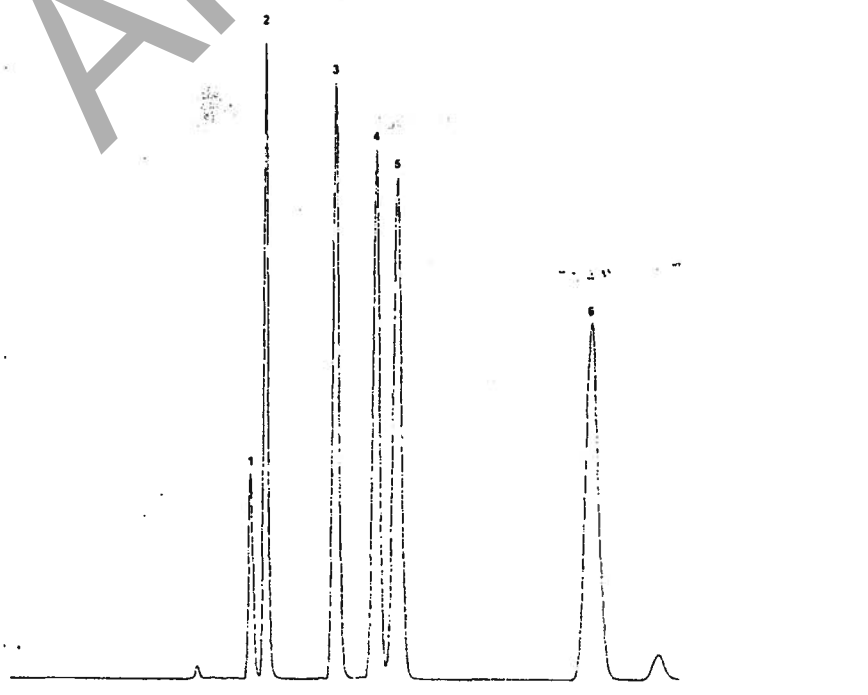
Peak Height Ratio:
NOT APPLICABLE

Resolution:
NOT APPLICABLE

Compound Identification	Retention Time (t_R)	Partition Ratio (k)	Peak Width ($W_{\frac{1}{2}}$)
1. ACETALDEHYDE	0.84	0.3	0.017
2. METHANOL	0.89	0.4	0.016
3. ETHANOL	1.14	0.7	0.023
4. ACETONE	1.29	1.0	0.026
5. ISOPROPANOL	1.37	1.1	0.032
6. 1-PROPANOL	2.06	2.1	0.049

(t_0)

Test Temperature: 40 °C
Carrier Gas: (He) 75.9 $\frac{cm}{sec}$ (10.1 $\frac{m}{m}$
Injection: Split Analyst: OLI



6/14/2004 BKL

BKL 5/27/05

APPENDIX D:GC Headspace Methods:

36 pages

Approved:

Barry K. Logan, PhD

Date: 8/5/04

Method Information

Method Change History

Operator	Date	Change Information
PAT FRIEL	11/30/99 4:13:57 PM	
PAT FRIEL	12/1/99 7:09:39 AM	
PAT FRIEL	12/1/99 7:10:13 AM	
M PEMBERTON	12/1/99 7:30:00 AM	
M PEMBERTON	12/1/99 7:58:19 AM	
M PEMBERTON	2/14/00 10:27:41 AM	
DR. MARTIN HUGHES	4/27/00 5:29:57 PM	
Estuardo J. Miranda	6/25/02 9:54:54 AM	
M PEMBERTON	4/30/2004 1:28:27 PM	
M PEMBERTON	4/30/2004 1:29:02 PM	
M PEMBERTON	4/30/2004 2:36:22 PM	
M PEMBERTON	5/3/2004 6:44:08 AM	
M PEMBERTON	5/3/2004 7:09:11 AM	
Bill	5/10/2004 7:08:46 AM	
Dora Schranz	6/3/2004 1:24:12 PM	
Dora Schranz	6/7/2004 9:07:06 AM	
N Nuwayhid, PhD	6/14/2004 4:56:13 PM	
N Nuwayhid, PhD	6/14/2004 4:59:22 PM	
' uwayhid, PhD	6/14/2004 5:12:54 PM	
. y wilson	6/15/2004 1:05:54 PM	
Estuardo J. Miranda	6/19/2004 1:46:32 PM	
Jayne E. Thatcher	6/21/2004 5:26:26 PM	
Kari Gruendell	6/24/2004 1:18:48 PM	
WP MARSHALL	7/1/2004 2:33:11 PM	
Dora Schranz	7/20/2004 6:42:12 AM	

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	on
Macro Name:	macro "contres4.mac",go
Save GLP Data:	off
Post-Run Cmd/Macro:	off
Save Method with Data:	on

Injection Source and Location

Injection Source: Manual

Injection Location: Front

6890 GC METHOD

OVEN
- Initial temp: 40 'C (On)
- Initial time: 2.20 min
- Ramps:

#	Rate	Final temp	Final time
1	0.0(Off)		

- Post temp: 70 'C
- Post time: 0.00 min
- Run time: 2.20 min
- Maximum temp: 280 'C
- Equilibration time: 0.50 min

FRONT INLET (UNKNOWN)
- Mode: Split
- Initial temp: 250 'C (On)
- Pressure: 10.22 psi (On)
- Split ratio: 1:1
- Split flow: 16.6 mL/min
- Total flow: 36.7 mL/min
- Gas saver: Off
- Gas type: Helium

BACK INLET ()

COLUMN 1
- Capillary Column
- Model Number: Agilent 125-9134
- DB-ALC1
- Max temperature: 280 'C
- Nominal length: 30.0 m
- Nominal diameter: 530.00 um
- Nominal film thickness: 3.00 um
- Mode: constant flow
- Initial flow: 16.6 mL/min
- Nominal init pressure: 10.23 psi
- Average velocity: 98 cm/sec
- Inlet: Front Inlet
- Outlet: Front Detector
- Outlet pressure: ambient

COLUMN 2
(not installed)

FRONT DETECTOR (FID)
- Temperature: 250 'C (On)
- Hydrogen flow: 40.0 mL/min (On)
- Air flow: 300.0 mL/min (On)
- Mode: Constant column+makeup flow
- Combined flow: 30.0 mL/min
- Makeup flow: On
- Makeup Gas Type: Nitrogen
- Flame: On
- Electrometer: On
- Lit offset: 2.0

BACK DETECTOR (NO DET)

SIGNAL 1
- Data rate: 5 Hz
- Type: front detector
- Save Data: On
- Zero: 0.0 (Off)
- Range: 0
- Fast Peaks: Off
- Attenuation: 0

SIGNAL 2
- Data rate: 20 Hz
- Type: front detector
- Save Data: Off
- Zero: 0.0 (Off)
- Range: 0
- Fast Peaks: Off
- Attenuation: 0

COLUMN COMP 1
Derive from front detector

COLUMN COMP 2
Derive from front detector

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

GC Injector

Front Injector:
Injector not configured, use these parameters if it becomes configured

Sample Washes	0
Sample Pumps	0
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
PostInj Solvent A Washes	0
PostInj Solvent B Washes	0
Viscosity Delay	0 seconds
Plunger Speed	Fast

Back Injector:
Injector not configured, use these parameters if it becomes configured

Sample Washes	0
Sample Pumps	0
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
PostInj Solvent A Washes	0
PostInj Solvent B Washes	0
Viscosity Delay	0 seconds
Plunger Speed	Fast

HEADSPACE PARAMETERS

Device:	Agilent G1888 Headspace Sampler
Comm:	IP|10.10.10.2
SN:	IT40320053
Vial Size:	10
GCHandshake Mode:	WAIT_H
Oven Stabilization Time:	1
Pressure Unit:	psi
Carrier Conn:	MANUAL_FRONT
Vial EPC:	NONE

Multi HS Extr:	OFF
Extractions Per Vial:	2
GC Cycle Time (Min):	2.4
Inject Time (Min):	0.16999999999999998
Loop Equilibration Time (Min):	0.15
Loop Fill Time (Min):	0.15
Loop Temperature:	85
Oven Temperature:	70
Shake:	LOW
Transfer Line Temperature:	90
Vial Equilibration Time (Min):	10
Vial Pressurization Time (Min):	0.16999999999999998

Headspace Pressures

Carrier:	0 psi
Vial:	0 psi

Integration Events

Results will be produced with the enhanced integrator.

Default Integration Event Table "Event"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_TCD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ADC"

Event	Value	Time
Initial Slope Sensitivity	20.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ECD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_NPD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FPD"

Event	Value	Time
Initial Slope Sensitivity	50.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_uECD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

BKL

Detector Default Integration Event Table "Event_FID"

Event	Value	Time
Initial Slope Sensitivity	50.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	20.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Apply Manual Integration Events: No

Advanced Baseline : No

Calibration Table

Calib. Data Modified : Wednesday, July 21, 2004 12:55:00 PM

Calculate : Internal Standard
Based on : Peak Area

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.050 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.050 min
Use Multiplier & Dilution Factor with ISTDs
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Floating Average New 75%
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Default Sample ISTD Information (if not set in sample table):

ISTD #	ISTD Amount [ng/ul]	Name
1	1.00000	n-Propanol

Signal 1: FID1 A,

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
1.053	1	1	7.90000e-2	398.14575	1.98420e-4	1		Ethanol
		2	1.58000e-1	823.35229	1.91898e-4			
		3	3.16000e-1	1642.63440	1.92374e-4			
1.693	1	1	1.00000	1290.18909	7.75080e-4	I1		n-Propanol

Method: D:\HPCHEM\1\METHODS\BLDALCO.M of 7/20/2004 6:42:12 AM

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
		2	1.00000	1316.01807	7.59868e-4			
		3	1.00000	1323.64648	7.55489e-4			

Peak Sum Table

No Entries in table

Method Information

Method Change History

Operator	Date	Change Information
PAT FRIEL	11/30/99 3:47:22 PM	
PAT FRIEL	11/30/99 4:20:27 PM	
PAT FRIEL	12/1/99 7:07:42 AM	
ANN MARIE GORDON	12/2/99 6:16:32 AM	
ED FORMOSO	12/4/99 9:53:18 AM	
PAT FRIEL	12/4/99 10:08:15 AM	
PAT FRIEL	12/4/99 10:23:35 AM	
PAT FRIEL	12/4/99 10:38:54 AM	
PAT FRIEL	12/6/99 7:38:51 AM	
PAT FRIEL	12/6/99 7:53:52 AM	
EGLE WEISS	12/9/99 2:04:47 PM	
M PEMBERTON	2/14/00 10:27:27 AM	
ED FORMOSO	2/22/00 7:37:01 AM	
G. Spencer	4/30/2004 9:16:12 AM	
G. Spencer	4/30/2004 9:18:00 AM	
G. Spencer	4/30/2004 1:34:08 PM	
m pemberton	5/3/2004 7:11:07 AM	
m pemberton	5/3/2004 9:08:35 AM	
r emberton	5/3/2004 9:40:07 AM	
emberton	5/3/2004 10:08:09 AM	
m pemberton	5/3/2004 10:39:00 AM	
m pemberton	5/3/2004 12:05:53 PM	
m pemberton	5/3/2004 12:25:00 PM	
m pemberton	5/3/2004 1:11:13 PM	
m pemberton	5/3/2004 1:34:47 PM	
m pemberton	5/3/2004 2:02:10 PM	
m pemberton	5/3/2004 2:23:37 PM	
m pemberton	5/3/2004 2:42:03 PM	
m pemberton	5/3/2004 3:08:52 PM	
m pemberton	5/3/2004 3:15:50 PM	
m pemberton	5/4/2004 6:39:02 AM	
m pemberton	5/4/2004 7:04:28 AM	
mp	5/6/2004 10:04:41 AM	
mp	5/6/2004 11:44:52 AM	
mp	5/6/2004 12:11:51 PM	
Bill	5/10/2004 7:06:02 AM	
mp	5/12/2004 6:49:50 AM	
mp	5/12/2004 7:11:03 AM	
b capron	6/8/2004 9:31:02 AM	
mary wilson	6/15/2004 1:01:34 PM	
Estuardo J. Miranda	6/19/2004 1:45:59 PM	

Run Time Checklist

Pre-Run Cmd/Macro: off

Data Acquisition: on

Standard Data Analysis: on

Customized Data Analysis: on
Macro Name: macro "contres5.mac",go

Save GLP Data: off

Post-Run Cmd/Macro: off

Save Method with Data: off

Injection Source and Location

Injection Source: Manual

Injection Location: Front

==========

6890 GC METHOD

==========

OVEN
Initial temp: 40 'C (On)
Initial time: 2.20 min
Ramps:
Rate Final temp Final time
1 0.0(Off)
Post temp: 70 'C
Post time: 0.00 min
Run time: 2.20 min

Maximum temp: 280 'C
Equilibration time: 0.50 min

FRONT INLET (UNKNOWN)
Mode: Split
Initial temp: 250 'C (On)
Pressure: 10.11 psi (On)
Split ratio: 1:1
Split flow: 16.7 mL/min
Total flow: 36.3 mL/min
Gas saver: Off
Gas type: Helium

BACK INLET ()

COLUMN 1
Capillary Column
Model Number: Agilent 125-9234
DB-ALC2
Max temperature: 280 'C
Nominal length: 30.0 m
Nominal diameter: 530.00 um
Nominal film thickness: 2.00 um
Mode: constant flow
Initial flow: 16.7 mL/min
Nominal init pressure: 10.11 psi
Average velocity: 98 cm/sec
Inlet: Front Inlet
Outlet: Front Detector
Outlet pressure: ambient

COLUMN 2
(not installed)

FRONT DETECTOR (FID)

BACK DETECTOR (NO DET)

Temperature: 250 'C (On)
Hydrogen flow: 40.0 mL/min (On)
Air flow: 450.0 mL/min (On)
Mode: Constant column+makeup flow
Combined flow: 30.0 mL/min
Makeup flow: On
Makeup Gas Type: Nitrogen
Flame: On
Electrometer: On
Lit offset: 2.0

SIGNAL 1
Data rate: 20 Hz
Type: front detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: front detector
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
Derive from front detector

COLUMN COMP 2
Derive from front detector

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

GC Injector

Front Injector:
Injector not configured, use these parameters if it becomes configured

Sample Washes	0
Sample Pumps	0
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
PostInj Solvent A Washes	0
PostInj Solvent B Washes	0
Viscosity Delay	0 seconds
Plunger Speed	Fast

Back Injector:
Injector not configured, use these parameters if it becomes configured

Sample Washes	0
Sample Pumps	0
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
PostInj Solvent A Washes	0
PostInj Solvent B Washes	0
Viscosity Delay	0 seconds
Plunger Speed	Fast

HEADSPACE PARAMETERS

Device:	Agilent G1888 Headspace Sampler
Comm:	IP|10.10.10.5
SN:	IT40320076
Vial Size:	10
GCHandshake Mode:	NO
Oven Stabilization Time:	1
Pressure Unit:	psi
Carrier Conn:	MANUAL_FRONT
Vial EPC:	NONE

Multi HS Extr:	OFF
Extractions Per Vial:	2
GC Cycle Time (Min):	2.4
Inject Time (Min):	0.16999999999999998
Loop Equilibration Time (Min):	0.15
Loop Fill Time (Min):	0.15
Loop Temperature:	85
Oven Temperature:	70
Shake:	LOW
Transfer Line Temperature:	90
Vial Equilibration Time (Min):	10
Vial Pressurization Time (Min):	0.16999999999999998

Headspace Pressures

Carrier:	0 psi
Vial:	0 psi

Integration Events

Results will be produced with the enhanced integrator.

Default Integration Event Table "Event"

Event	Value	Time
Initial Slope Sensitivity	1.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.700	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_TCD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ADC"

Event	Value	Time
Initial Slope Sensitivity	20.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_ECD"

Event	Value	Time
Initial Slope Sensitivity	100.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_NPD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FPD"

Event	Value	Time
Initial Slope Sensitivity	50.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_uECD"

Event	Value	Time
Initial Slope Sensitivity	500.000	Initial
Initial Peak Width	0.080	Initial
Initial Area Reject	1.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Detector Default Integration Event Table "Event_FID"

Event	Value	Time
Initial Slope Sensitivity	75.000	Initial
Initial Peak Width	0.040	Initial
Initial Area Reject	20.000	Initial
Initial Height Reject	1.000	Initial
Initial Shoulders	OFF	Initial

Apply Manual Integration Events: No

Advanced Baseline : No

Calibration Table

Calib. Data Modified : Wednesday, July 21, 2004 12:54:16 PM

Calculate : Internal Standard
Based on : Peak Area

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.050 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.050 min
Use Multiplier & Dilution Factor with ISTDs
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Floating Average New 75%
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Default Sample ISTD Information (if not set in sample table):

ISTD #	ISTD Amount [ng/ul]	Name
1	1.00000	n-Propanol

Signal 1: FID1 A,

RetTime [min]	Lvl Sig	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
1.104	1	1 7.90000e-2	409.98511	1.92690e-4	1		Ethanol
		2 1.58000e-1	805.75922	1.96088e-4			
		3 3.16000e-1	1562.12549	2.02288e-4			
1.914	1	1 1.00000	1277.35852	7.82866e-4	I1		n-Propanol

Method: D:\HPCHEM\1\METHODS\BLDALCO2.M of 6/19/2004 1:45:59 PM

RetTime [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
		2	1.00000	1249.28589	8.00457e-4			
		3	1.00000	1230.50989	8.12671e-4			

Peak Sum Table

No Entries in table

Sample Alcohol Chromoatogram:

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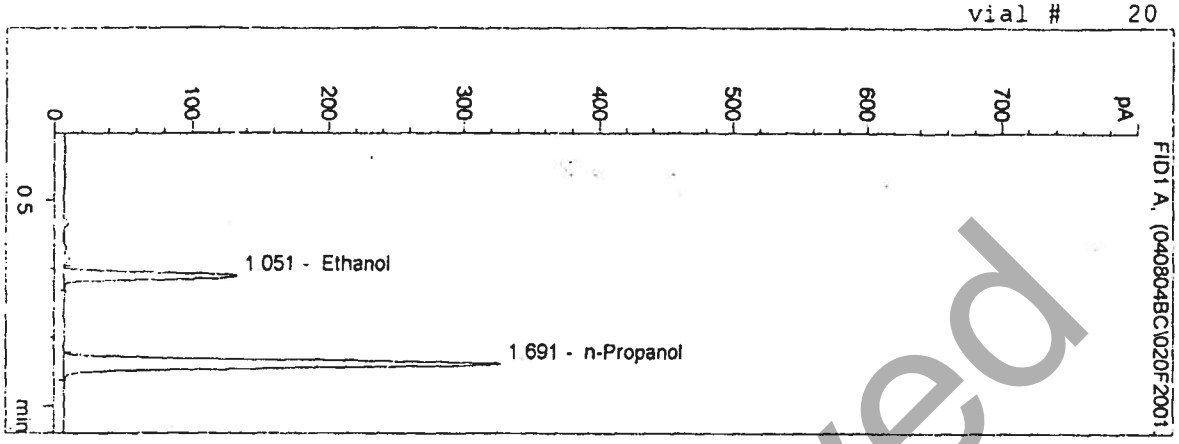
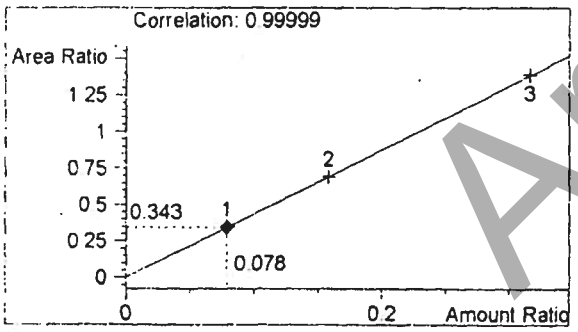
D:\HPCHEM\1\METHODS\BLDALCO.M
8/4/2004 3:40:00 PM 045234
Instrument 4 brian capron
DB-ALC1

vial # 20

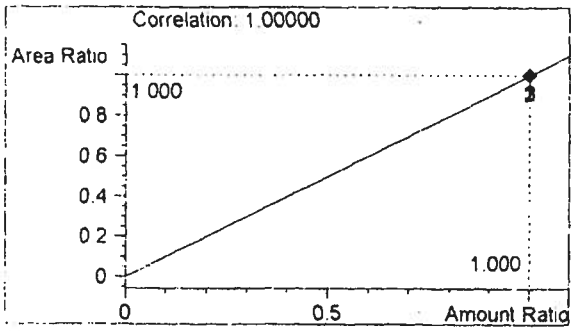


#	Compound	Area	RT
1	Ethanol	424	1.051
2	n-Propanol	1237	1.691

Totals:



Ethanol 0.078 g/100ml



n-Propanol 1.000 g/100ml

Approved: _____
Barry K. Logan, PhD

Date: 8/5/04

Date	Analyst	QC - & Corrective Action

Approved:

Barry K. Logan, PhD

Date: 8/5/04

Volatile Method Calibrators

Approved:

Barry K. Logan, PhD

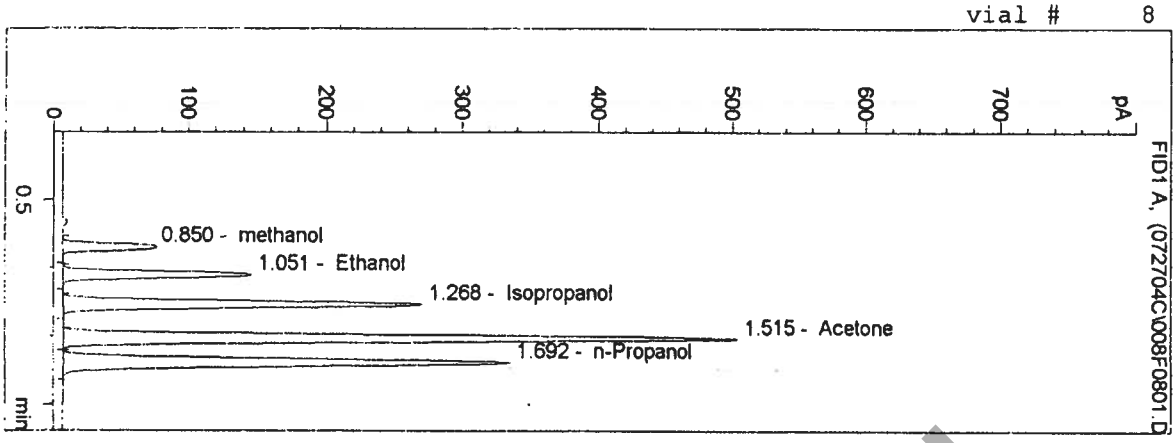
Date: 8/5/04

WASHINGTON STATE TOXICOLOGY LABORATORY

D:\HPCHEM\1\METHODS\VOL.M
7/27/2004 10:28:45 AM
Instrument 4
ALC1

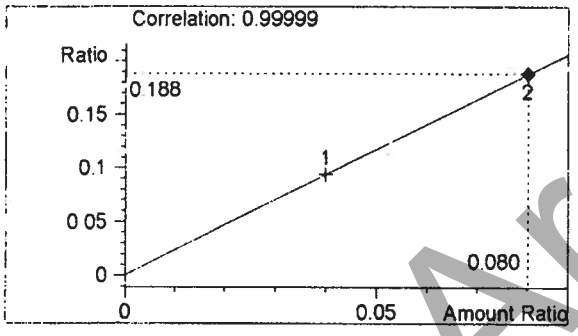
0.08 mix std
M PEMBERTON

vial # 8

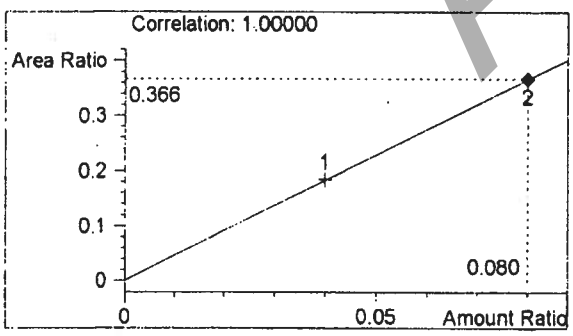


#	Compound	Area	RT
1	methanol	237	0.850
2	Ethanol	461	1.051
3	Isopropanol	906	1.268
4	Acetone	1738	1.515
5	n-Propanol	1260	1.692

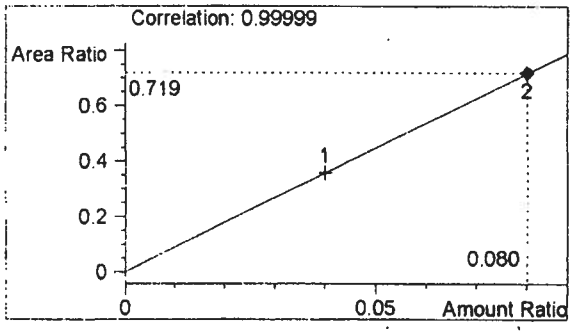
Totals:



methanol 0.080 g/100ml



Ethanol 0.080 g/100ml



Isopropanol 0.080 g/100ml

BKL

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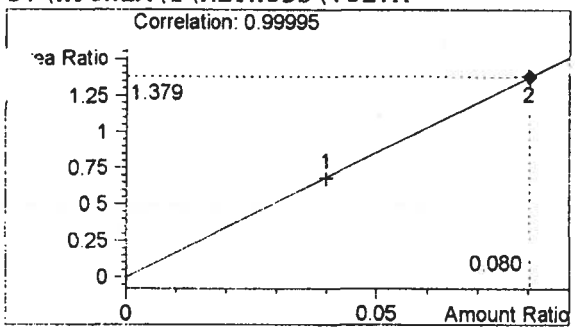
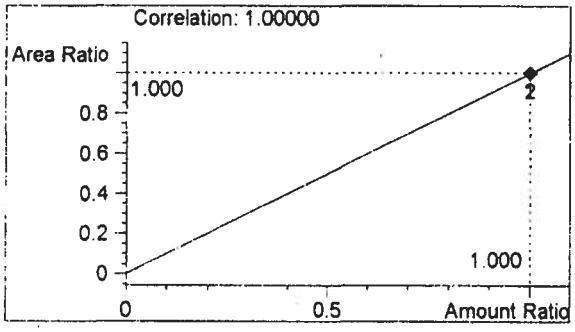
D:\HPCHEM\1\METHODS\VOL.M



Acetone 0.080 g/100ml



n-Propanol 1.000 g/100ml

BLL

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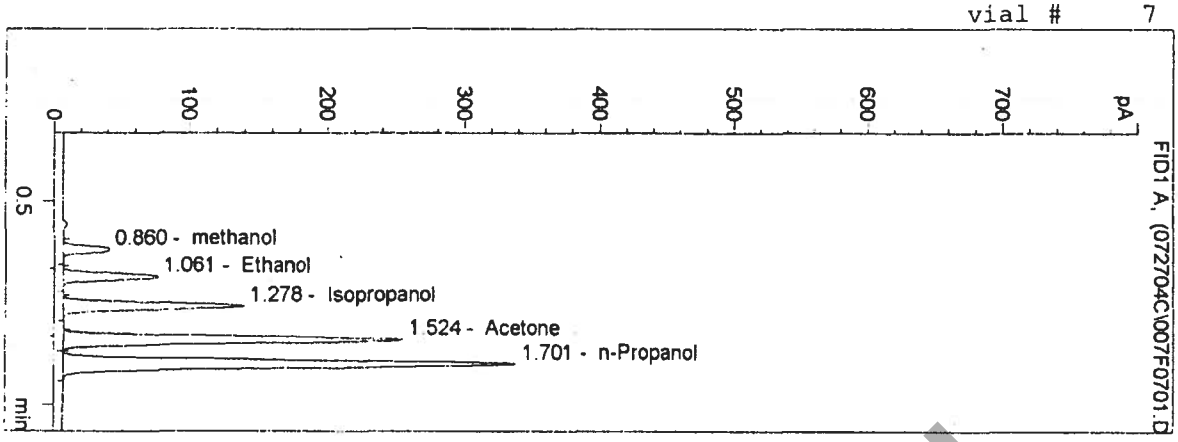
D:\HPCHEM\1\METHODS\VOL.M
7/27/2004 10:25:34 AM
Instrument 4
ALC1

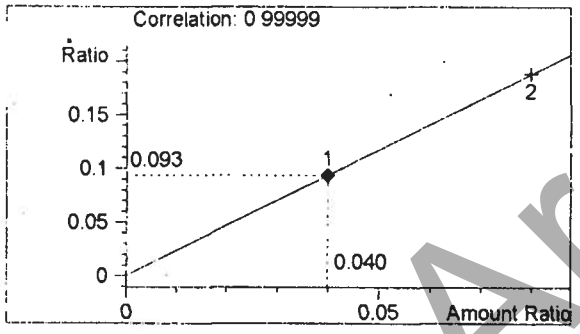
0.04 mix std
M PEMBERTON

vial # 7

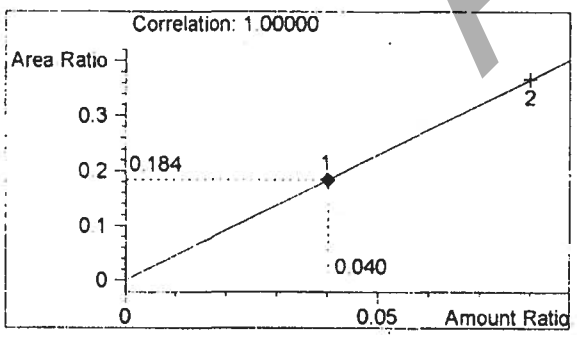


#	Compound	Area	RT
1	methanol	120	0.860
2	Ethanol	235	1.061
3	Isopropanol	458	1.278
4	Acetone	868	1.524
5	n-Propanol	1281	1.701

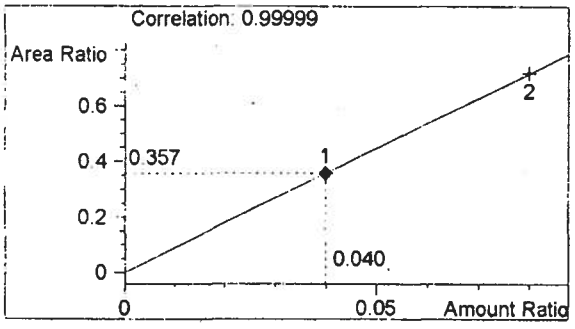
Totals:



methanol 0.040 g/100ml



Ethanol 0.040 g/100ml



Isopropanol 0.040 g/100ml

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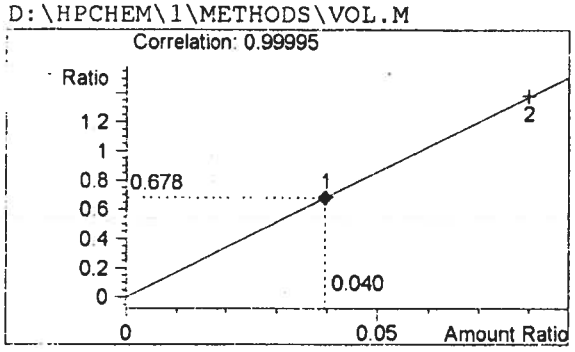
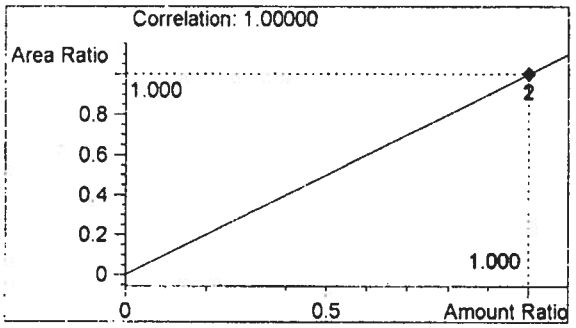
D:\HPCHEM\1\METHODS\VOL.M



Acetone 0.040 g/100ml



n-Propanol 1.000 g/100ml

BKL

Volatiles and Relative Retention Times

Retention Times of Common Volatiles								
Instrument	#1		#3		#4		#5	
Updated	Mar-04		Mar-04		Jun-04		Jun-04	
	DBA1c-1	RRT	DBA1c-2	RRT	DBA1c-1	RRT	DBA1c-2	
n-Propanol (ISTD)	1.700		1.856		1.720		1.901	
Tetrafluroethane	0.691	0.406	0.680	0.366	0.747	0.434	0.655	0.345
Difluroethane	0.718	0.422	0.691	0.372	0.722	0.420	0.667	0.351
Desflurane	0.827	0.486	0.805	0.434	0.790	0.459	0.876	0.461
Methanol	0.829	0.488	0.878	0.473	0.850	0.494	0.875	0.468
Ethanol	1.041	0.612	1.091	0.588	1.050	0.610	1.097	0.576
Sevoflurane	1.162	0.684	1.069	0.576	1.201	0.698	1.073	0.564
Diethyl Ether	1.250	0.735	0.979	0.527	1.284	0.747	0.972	0.511
Isopropanol	1.266	0.745	1.279	0.689	1.270	0.738	1.291	0.677
Isoflurane	1.291	0.759	1.269	0.684	1.327	0.772	1.285	0.676
Enflurane	1.442	0.848	1.374	0.740	1.474	0.857	1.394	0.733
Acetonitirle	1.517	0.892	1.455	0.784	1.539	0.895	1.472	0.774
Acetone	1.522	0.895	1.206	0.650	1.517	0.882	1.215	0.637
Halothane	1.726	1.015	1.679	0.905	na		1.709	0.899
MEK	2.800	1.647	2.080	1.121	2.854	1.659	2.130	1.120

Indicates RT difference for ISTD

Approved:

Barry K. Logan, PhD

Date: 8/5/04