

VERMONT FORENSIC LABORATORY

Verification  
BLOOD ALCOHOL DATA REVIEW CHECKLIST

5/19/15  
AGENCY CASE#: FN08101401  
LAB #: N/A

ANALYST: AB

TECHNICAL REVIEWER: [Signature]

DATE: 5/21/2015

- QC complete?
- Pertinent standard lot numbers listed?
- Data package is complete?
- Transcribed data and calculations verified?
- Calibration and control results all within  $\pm 10\%$  ( $\pm 20\%$  for STD A) of the known values?
- Calibration line correlation factor at least 0.99xx?
- Average error for components of the calibration line less than 10%?
- Timing mix chromatogram demonstrates appropriate identification of components and separation from ethanol?
- Surrogate compound concentrations all within 0.900 to 1.100 range?
- Statistical means of the continuing calibration check sample duplicate analysis all within  $\pm 10\%$  of the known value?
- Individual replicates within  $\pm 5\%$  of the statistical mean?
- Reported results are means of two or more analyses?
  1. In the event of QNS for 2 (two) analysis a notation must be made on the worksheet.
  2. In the event that more than 2 (two) results are used to determine the reported result a notation is made on the worksheet.

Comments:

ADMINISTRATIVE REVIEWER: TA

DATE: 5/26/2015

- Agency Case # and Lab # and Evidence description match submission form?
- Submitting agency, investigator, suspect, CC all match submission form?
- Chain of Custody complete and dated appropriately?
- Are all pages numbered and initialed on the Examination Documents?
- Case number (agency or lab number) written on all pages of the case file? unique #
- Appropriate review(s) have been completed?
- Is the exam end date documented in the paper case file consistent with the exam end date in LIMS?

Comments:

DIRECTOR REVIEWER: TA

DATE: 5/26/2015

- Does the report and associated paperwork meet administrative expectations?
- Have the appropriate reviews been completed?

Check Mark or X = pass, NA= Not Applicable

*Certificate of Analysis*  
*Certified Reference Standard - NIST Traceable*

**Ethanol-20**  
*Ethyl Alcohol*

Cerilliant Quality
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 15194
ISO 9001
GMP/GLP

**Catalog Number:** E-056  
**Solution Lot:** FN08101401  
**Expiration Date:** November 2019  
**Diluent:** Water  
**Volume per Ampoule:** 1.2 mL  
**Storage:** Refrigerate. Do not freeze.  
**Intended Use:** For R&D/ analytical purposes only. Not suitable for human or animal consumption.

- Expiration Date has been established through real time stability studies and applies to the ampoule stored unopened at the recommended storage condition.
- Ampoules are overfilled to ensure a minimum 1.2 mL volume fill. We advise laboratories to use measured volumes of this standard solution before diluting to the desired concentration. The standard should be used immediately after opening to avoid concentration changes due to evaporation.
- For quantitative applications, the minimum sample size for intended use is 100 µL.

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	>99.9%	20.00 ± 0.07 mg/dL
<ul style="list-style-type: none"> <li>▪ Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.</li> <li>▪ When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).</li> <li>▪ The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.</li> <li>▪ Purity factor has been established through independent certification of the neat analyte to ISO 17025 standards – See page 2.</li> <li>▪ Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.</li> </ul>		

**Traceability to SI through NIST:**

- This standard has been prepared and certified under the ISO Guide 34 and ISO/IEC 17025 standards and meets the requirements of a Certified Reference Material as defined by ISO.
- Gravimetrically prepared using qualified balances calibrated semi-annually by Mettler Toledo to ISO 17025 requirements and using NIST traceable weights. Qualification of each balance includes the assignment of a minimum weighing by Mettler Toledo taking into consideration the balance and installed environmental conditions to ensure each weighing complies with USP tolerances of NMT 0.1% relative uncertainty.
- Balance calibration adjustments are performed weekly utilizing the balance's internal adjustment mechanism and with NIST traceable weights.
- Balance calibration is verified prior to each use and is performed utilizing NIST traceable weights. Weigh tapes from the balance calibration are included in the production batch record for this standard. Production data package available upon request.
- Fill volume is gravimetrically verified throughout the dispensing process using qualified balances calibrated with NIST traceable weights.
- Weight sets used for all balance calibrations are calibrated externally by an ISO 17025 accredited calibration laboratory to NIST standards.
- Concentration of this standard has been analytically verified against a NIST SRM and a Control using a validated method. See page 2.

Cerilliant certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration date. Warranty applies to ampoules stored unopened and stored under the recommended storage conditions. Warranty and expiry do not extend to solutions into which this product has been incorporated. Establishment of shelf life of all such products is the responsibility of the user.



Darron Ellsworth, Quality Assurance Manager

*December 29, 2014*  
Date

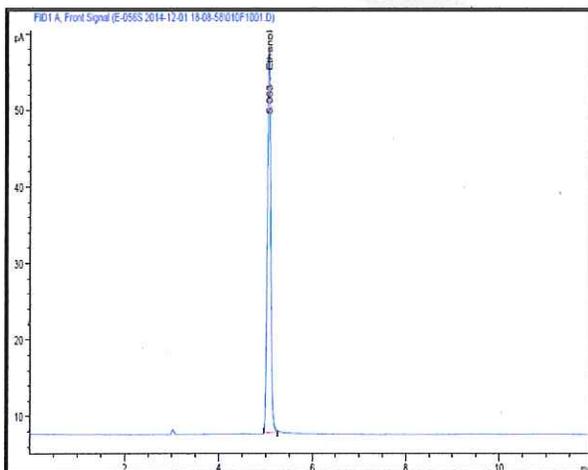
**Analytical Verification of Solution Standard Concentration and Batch Homogeneity:**

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2891 (mg/dL)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN08101401	20.03	0.84%
Prior Lot	FN09031301	20.07	0.62%
Acceptance Criteria		±2%	≤2%

- Concentration is calculated as the average of multiple analyses conducted using a validated Headspace GC/FID method. The validated GC/HS method has been demonstrated to adequately detect and quantitate ethanol concentrations ranging from 5 to 600 mg/dL. Relative standard uncertainty of the analysis is 1.675% and includes both uncertainty of the analytical method and uncertainty of the NIST SRM concentration.
- The Control is independently prepared from a different lot of neat ethanol to ensure no bias in the analysis and independently qualified against a NIST SRM.
- Homogeneity is ensured through rigorous production process controls statistically analyzed to evaluate risk and verified by analysis. The %RSD of samples pulled from across the lot using a stratified random sampling plan demonstrates ampoule to ampoule consistency or homogeneity of the New Lot.
- The %RSD of the Prior Lot represents system suitability on the date of analysis. Triplicate injections of the Prior Lot are bracketed at the beginning and end of the sequence. %RSD criteria ensures proper system performance throughout the sequence.
- All instruments used for certification of the neat materials and verification of the solution concentration and homogeneity are fully qualified through an Installation Qualification and an Operational Qualification which is repeated annually. System suitability is performed daily with rigorous acceptance criteria to ensure the system continues to perform within the validated parameters.

**Solution Standard Assay Parameters**

**Analysis Method:** GC/FID Headspace  
**Column:** DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness  
**Temp Program:** 40°C hold for 12 min  
**Injector Temp:** 200°C  
**Detector Temp:** 250°C



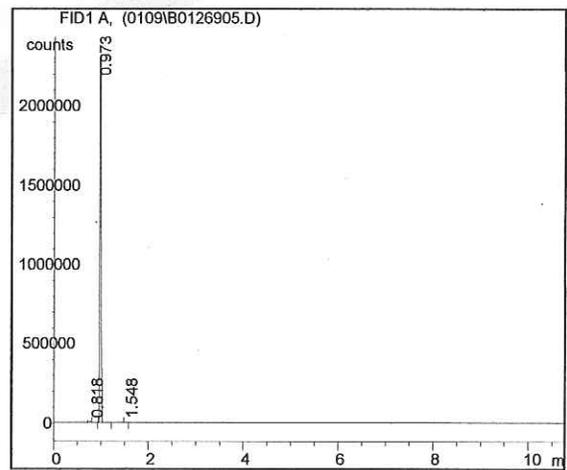
**Neat Material Analysis**

**Purity by GC/FID Analysis:** >99.9%

**Water Content by Karl Fischer:** 0.1%

**Purity Factor:** 99.9%

The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.



File Name = C:\Alcohol Data\VFL05202015\VFL05202015.SEQ

Version = 1

File Date = 5/20/2015 9:58:14 AM

Entry #	Raw File Name	Method File Name	Sample Name	Sample Wt.	Int. Std. Amount
1	VFL05202015.0001.raw	Blood Alc temp VFL.MET	Blank	1.00	1.00
2	VFL05202015.0002.raw	Blood Alc temp VFL.MET	TMX	1.00	1.00
3	VFL05202015.0003.raw	Blood Alc temp VFL.MET	Std A	1.00	1.00
4	VFL05202015.0004.raw	Blood Alc temp VFL.MET	Std B	1.00	1.00
5	VFL05202015.0005.raw	Blood Alc temp VFL.MET	Std C	1.00	1.00
6	VFL05202015.0006.raw	Blood Alc temp VFL.MET	Std D	1.00	1.00
7	VFL05202015.0007.raw	Blood Alc temp VFL.MET	Std E	1.00	1.00
8	VFL05202015.0008.raw	Blood Alc temp VFL.MET	WB	1.00	1.00
9	VFL05202015.0009.raw	Blood Alc temp VFL.MET	WB	1.00	1.00
10	VFL05202015.0010.raw	Blood Alc temp VFL.MET	CCS	1.00	1.00
11	VFL05202015.0011.raw	Blood Alc temp VFL.MET	CCS	1.00	1.00
12	VFL05202015.0012.raw	Blood Alc temp VFL.MET	V15-774 A1-1	1.00	1.00
13	VFL05202015.0013.raw	Blood Alc temp VFL.MET	V15-774 A1-1	1.00	1.00
14	VFL05202015.0014.raw	Blood Alc temp VFL.MET	V15-795 A1-1	1.00	1.00
15	VFL05202015.0015.raw	Blood Alc temp VFL.MET	V15-795 A1-1	1.00	1.00
16	VFL05202015.0016.raw	Blood Alc temp VFL.MET	FN08101401	1.00	1.00
17	VFL05202015.0017.raw	Blood Alc temp VFL.MET	FN08101401	1.00	1.00
18	VFL05202015.0018.raw	Blood Alc temp VFL.MET	CCS	1.00	1.00
19	VFL05202015.0019.raw	Blood Alc temp VFL.MET	CCS	1.00	1.00

Case work samples not included AS 5/21/15

Lot #'s

- Aqueous Blank - BL-01162015
  - Timing Mix - TMX 08112014
  - Internal Standard - IS-01132015
  - Aqueous Control - Ceilliant FN021111-03
  - Whole Blood Control - Cliniga 1212090
  - Ethanol Standards: Ceilliant
    - A FN01131401
    - B FN092710-01
    - C FN05211402
    - D FN032712-01
    - E FN012712-01
- AB 5/21/15

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File Name: C:\Methods\VFL05202015.CAL  
 Version: 8

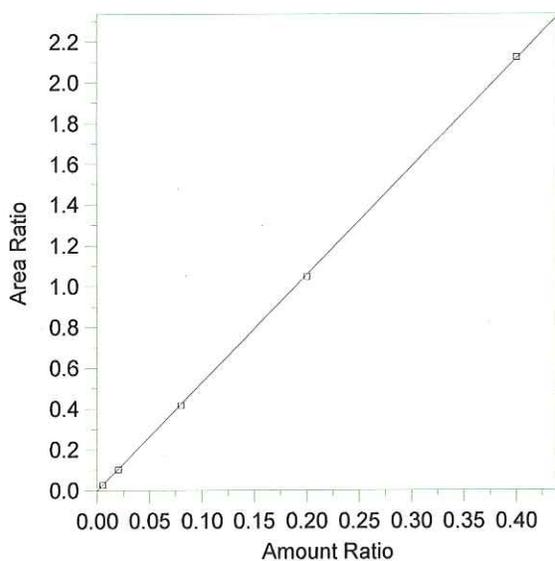
Creator:  
 Description:  
 Reason for change:

Internal standard calibration  
 No injection volume correction  
 No sample weight correction  
 Area reject threshold: 1500  
 Reference peak area reject threshold: 0  
 Amount units:  
 No default component

Method of calculating data point averages: Equal weight for all updates  
 No calibration update report

All levels are normal data points.

3 Ethanol



Expected retention time: 2.652 minutes  
 Search window: 0.1 minutes  
 Internal standard component: 7 (n-Propanol)  
 No retention time reference component  
 Group number: 0  
 High alarm limit: 0  
 Low alarm limit: 0  
 Component constant: 0  
 Single peak quantification by area  
 $Y = 5.287765 X + 0$   
 Linear fit with equal weighting, forced to origin  
 Coefficient of determination: 0.9999325  
 Average error: 1.478%  
 Average CF: 5.255792  
 RSD: 1.786%

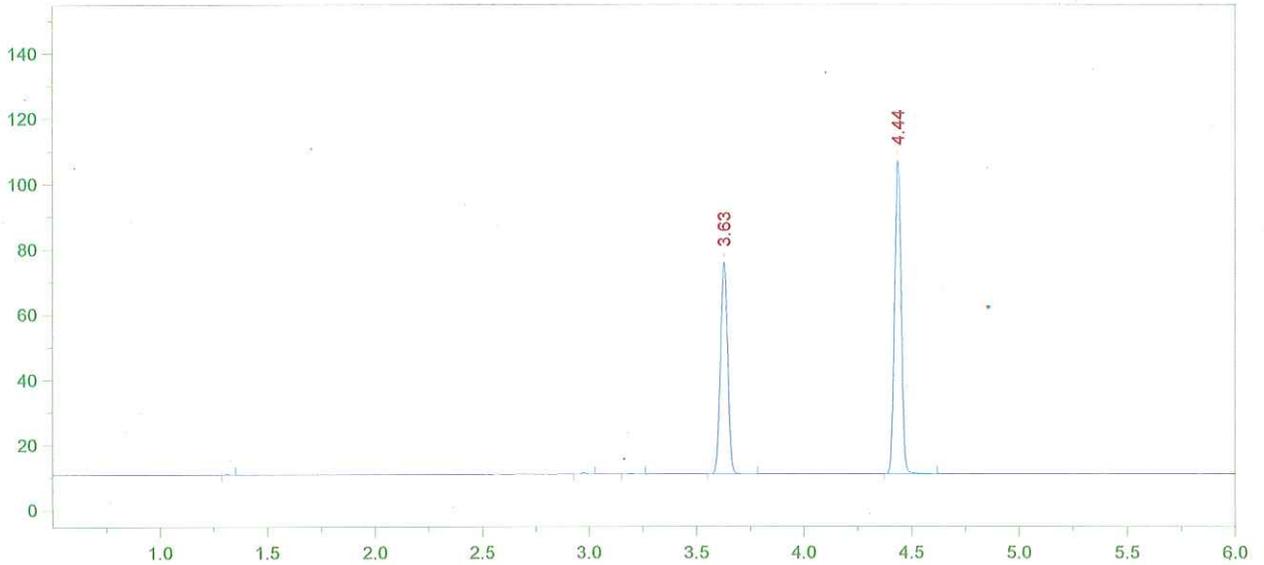
Level	Amount	Response	Cal Factor	Error, %	Amount Ratio	Response Ratio	Source	Date and time
1	(0.0836)	(85146)	--	--	0.0836	85146	Manual	8/13/2014 9:33:12 AM
2	0.005	5382.839	1076568	1.868	0.005	2.693264E-02	C:\Alcohol Data\VFL05202015\VFL05202015.0003.BND	5/20/2015 11:03:48 AM
3	0.02	18811.33	940566.5	-2.811	0.02	0.1027822	C:\Alcohol Data\VFL05202015\VFL05202015.0004.BND	5/20/2015 11:22:04 AM
4	0.08	89519.65	1118996	-1.378	0.08	0.417193	C:\Alcohol Data\VFL05202015\VFL05202015.0005.BND	5/20/2015 11:36:55 AM
5	0.2	190043.3	950216.5	-1.019	0.2	1.046781	C:\Alcohol Data\VFL05202015\VFL05202015.0006.BND	5/20/2015 11:53:29 AM
6	0.4	394836.7	987091.7	0.316	0.4	2.1218	C:\Alcohol Data\VFL05202015\VFL05202015.0007.BND	5/20/2015 12:07:52 PM

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
Bound File Name = VFL05202015.0001.RAW  
Sample Name = Blank  
Date Taken = 5/20/2015 10:33:22 AM  
Method file = Blood Alc temp VFL.MET  
Calibration file = VFL05202015.CAL

Today's Time = 8:26:09 AM

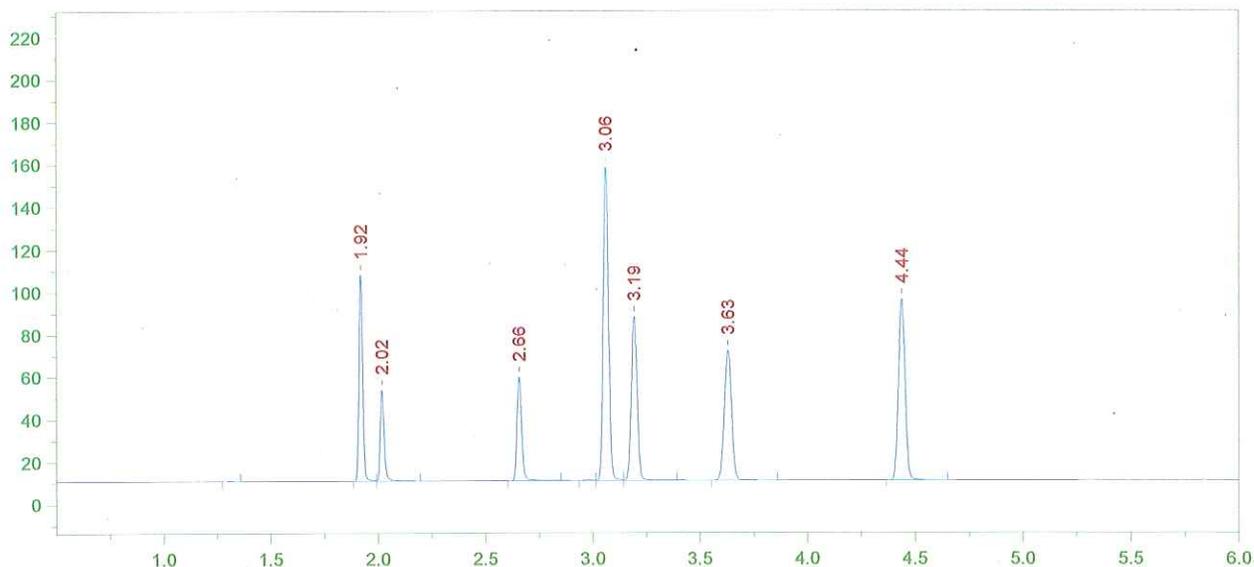
VFL Headspace Alcohol

Calibration Version = 8

Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
4	3.63	t-Butanol	150122	0.9625
5	4.44	n-Propanol	204689	1.0000

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Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
 Bound File Name = VFL05202015.0002.RAW  
 Sample Name = TMX  
 Date Taken = 5/20/2015 10:50:30 AM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 8:26:17 AM

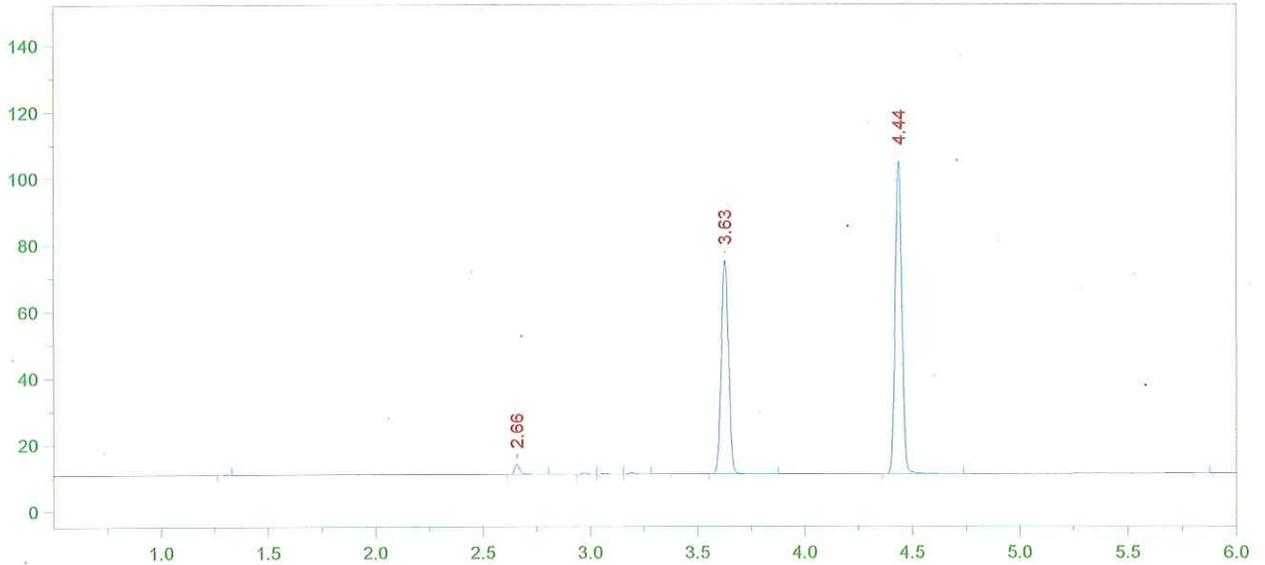
VFL Headspace Alcohol

Calibration Version = 8

Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	1.92	Acetaldehyde	113398	0.0695
3	2.02	Methanol	54816	0.1232
4	2.66	Ethanol	76115	0.0795
6	3.06	Acetone	253351	0.0657
7	3.19	Isopropanol	145430	0.0848
8	3.63	t-Butanol	141767	1.0276
9	4.44	n-Propanol	181052	1.0000

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Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
 Bound File Name = VFL05202015.0003.RAW  
 Sample Name = Std A  
 Date Taken = 5/20/2015 11:03:39 AM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 8:26:22 AM  
 VFL Headspace Alcohol  
 Calibration Version = 8

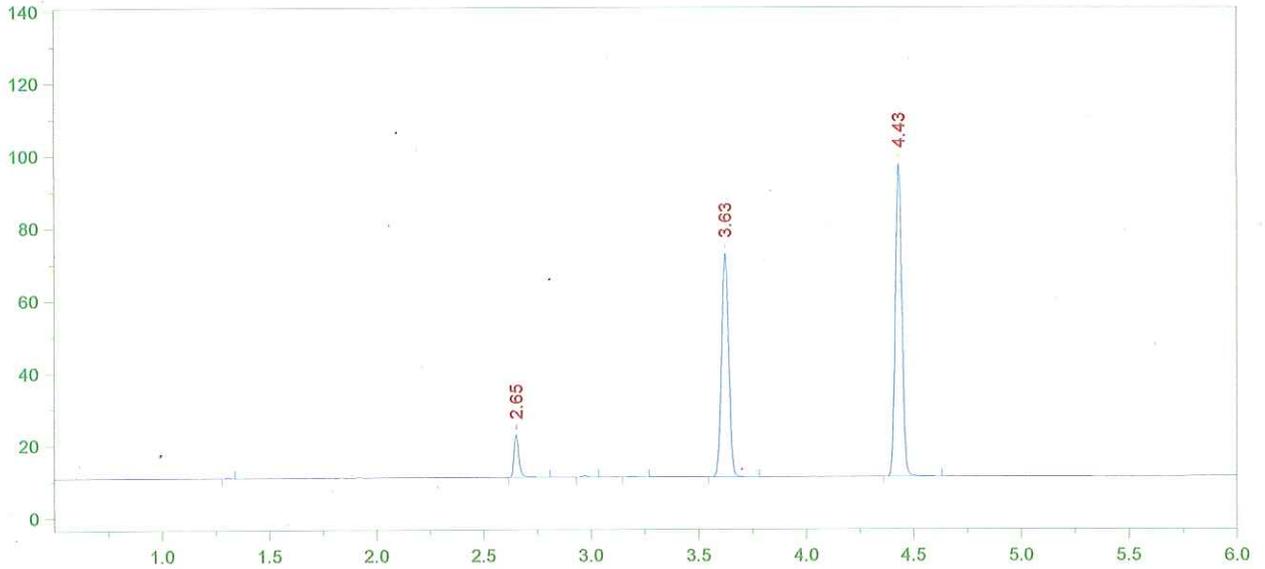
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.66	Ethanol	05383	0.0051
6	3.63	t-Butanol	149467	0.9814
7	4.44	n-Propanol	199863	1.0000

FN 08101401

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
 Bound File Name = VFL05202015.0004.RAW  
 Sample Name = Std B  
 Date Taken = 5/20/2015 11:21:57 AM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 8:26:28 AM

VFL Headspace Alcohol

Calibration Version = 8

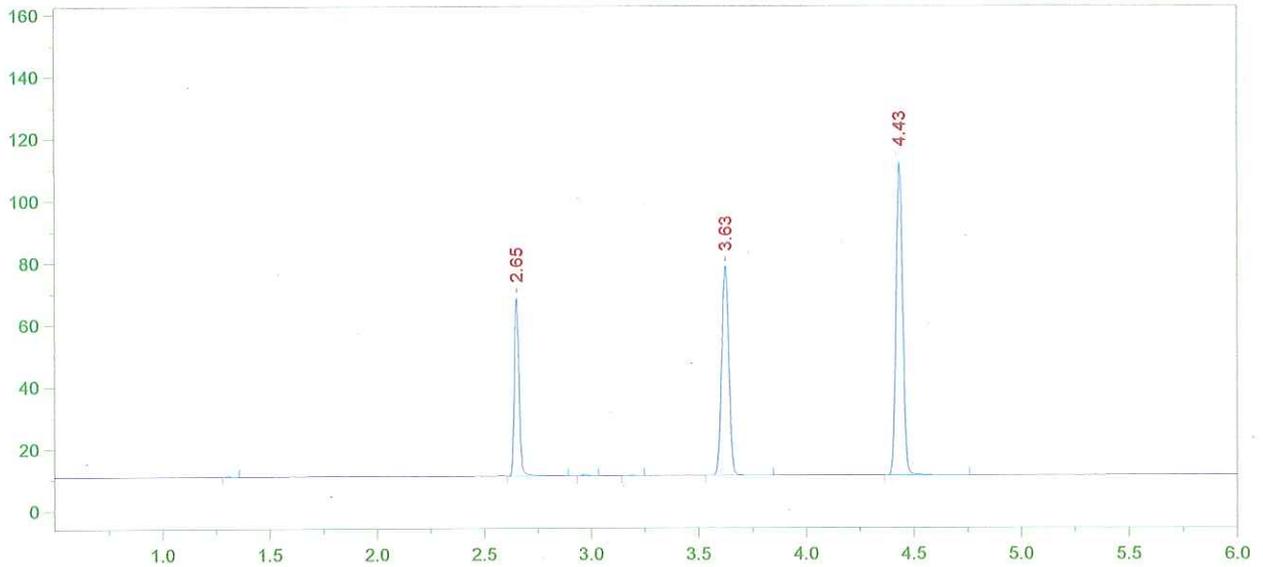
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	18811	0.0194
5	3.63	t-Butanol	143180	1.0266
6	4.43	n-Propanol	183021	1.0000

FN08101401

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
 Bound File Name = VFL05202015.0005.RAW  
 Sample Name = Std C  
 Date Taken = 5/20/2015 11:36:48 AM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 8:26:34 AM  
 VFL Headspace Alcohol  
 Calibration Version = 8

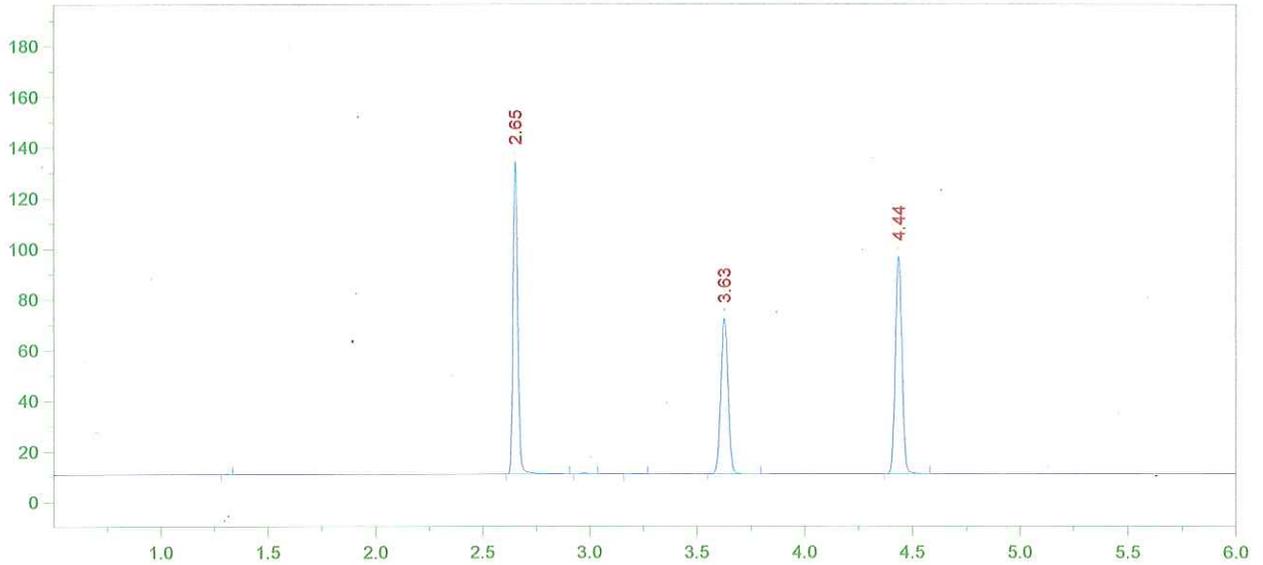
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	89520	0.0789
5	3.63	t-Butanol	157018	0.9603
6	4.43	n-Propanol	214576	1.0000

FN 08101401

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
 Bound File Name = VFL05202015.0006.RAW  
 Sample Name = Std D  
 Date Taken = 5/20/2015 11:53:24 AM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 8:26:39 AM  
 VFL Headspace Alcohol  
 Calibration Version = 8

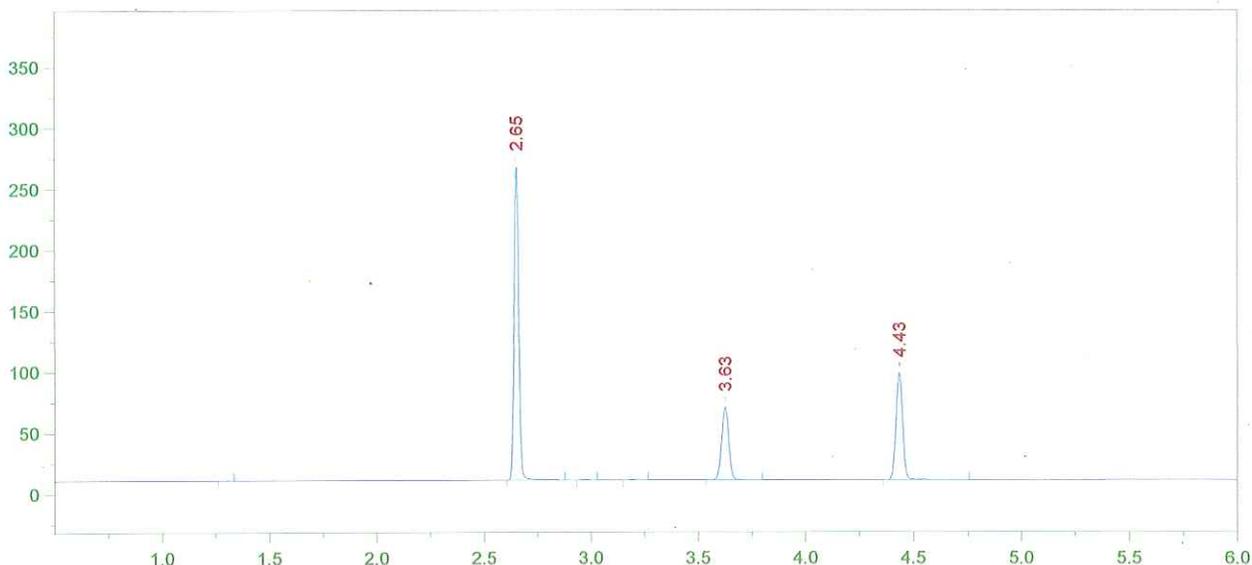
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	190043	0.1980
5	3.63	t-Butanol	142618	1.0309
6	4.44	n-Propanol	181550	1.0000

FN08101401

8

AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/21/2015  
 Bound File Name = VFL05202015.0007.RAW  
 Sample Name = Std E  
 Date Taken = 5/20/2015 12:07:47 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 8:26:45 AM

VFL Headspace Alcohol

Calibration Version = 8

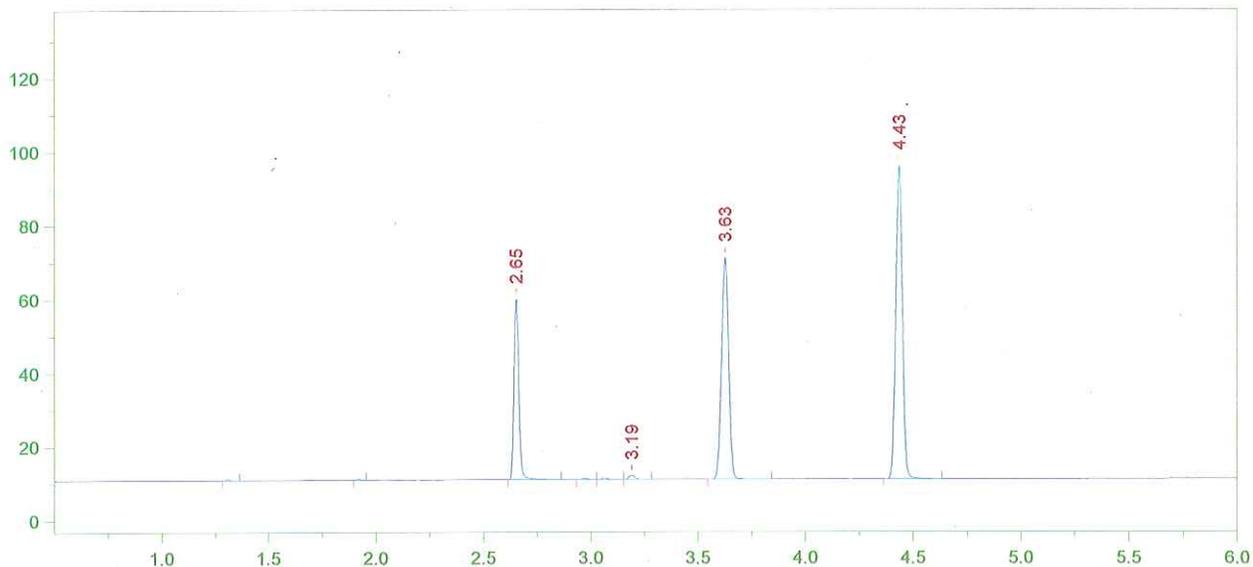
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	394837	0.4013
5	3.63	t-Butanol	137993	0.9732
6	4.43	n-Propanol	186086	1.0000

FN 08101401

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0008.RAW  
 Sample Name = WB  
 Date Taken = 5/20/2015 12:24:46 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 12:24:51 PM  
 VFL Headspace Alcohol  
 Calibration Version = 8

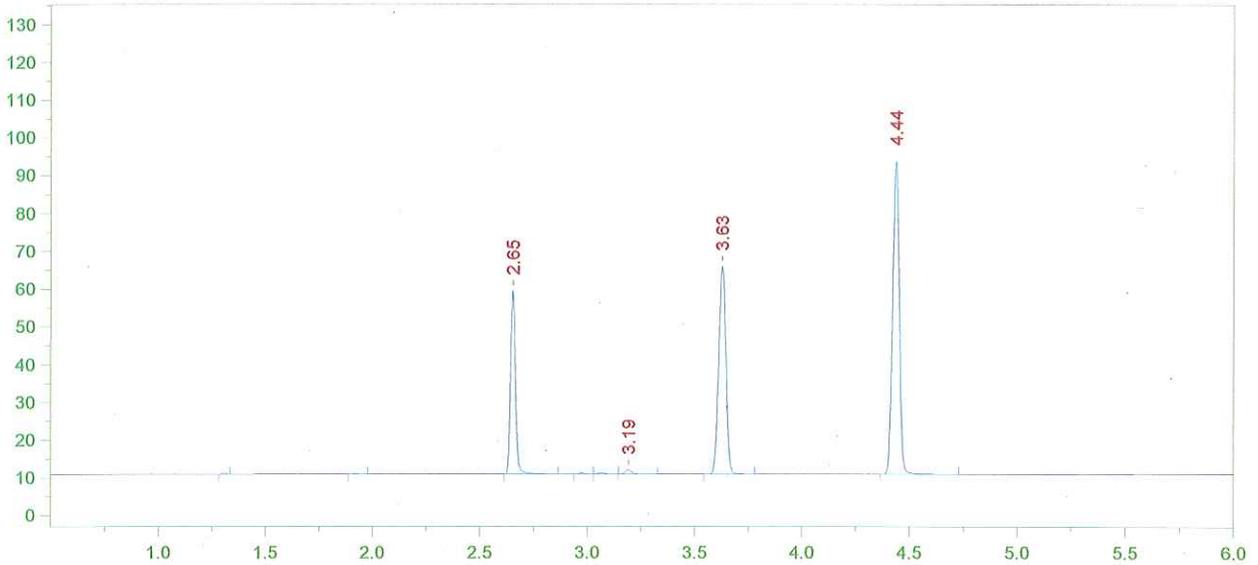
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
3	2.65	Ethanol	76672	0.0805
6	3.19	Isopropanol	02122	<0.004
7	3.63	t-Butanol	139425	1.0157
8	4.43	n-Propanol	180151	1.0000

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0009.RAW  
 Sample Name = WB  
 Date Taken = 5/20/2015 12:39:44 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 12:39:49 PM

VFL Headspace Alcohol

Calibration Version = 8

Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
3	2.65	Ethanol	75973	0.0814
6	3.19	Isopropanol	02171	<0.004
7	3.63	t-Butanol	127896	0.9508
8	4.44	n-Propanol	176528	1.0000

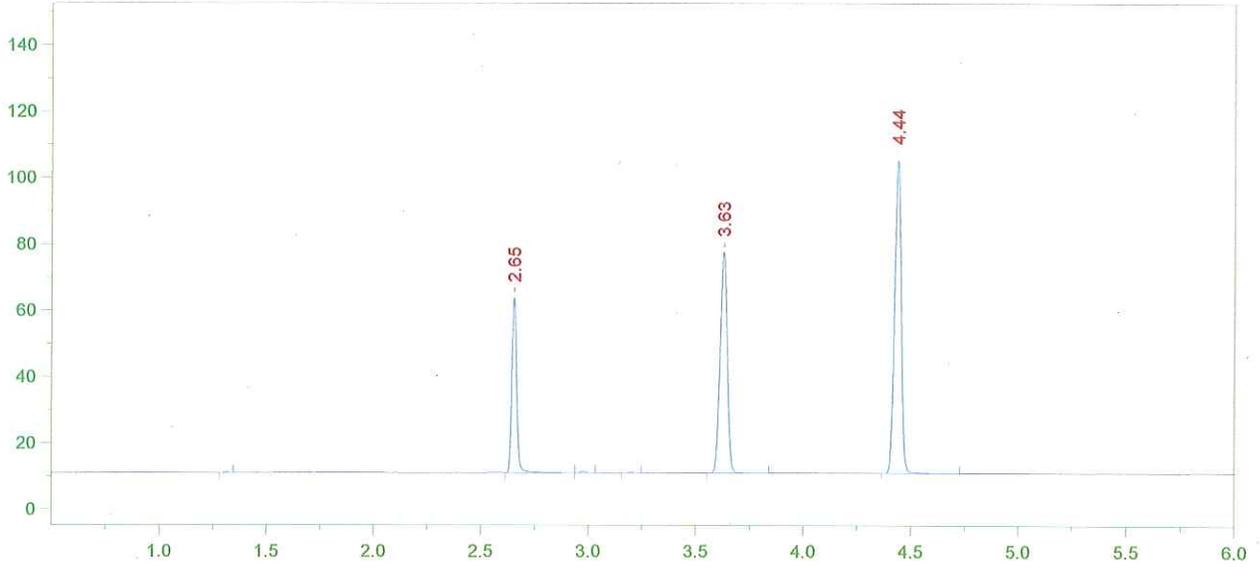
*Handwritten:* Avg vals 8+9 = 0.081  
 AB  
 5/21/15

*Handwritten:* FN08101401

*Handwritten:* 11

*Handwritten:* AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0010.RAW  
 Sample Name = CCS  
 Date Taken = 5/20/2015 12:56:10 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 12:56:16 PM

VFL Headspace Alcohol

Calibration Version = 8

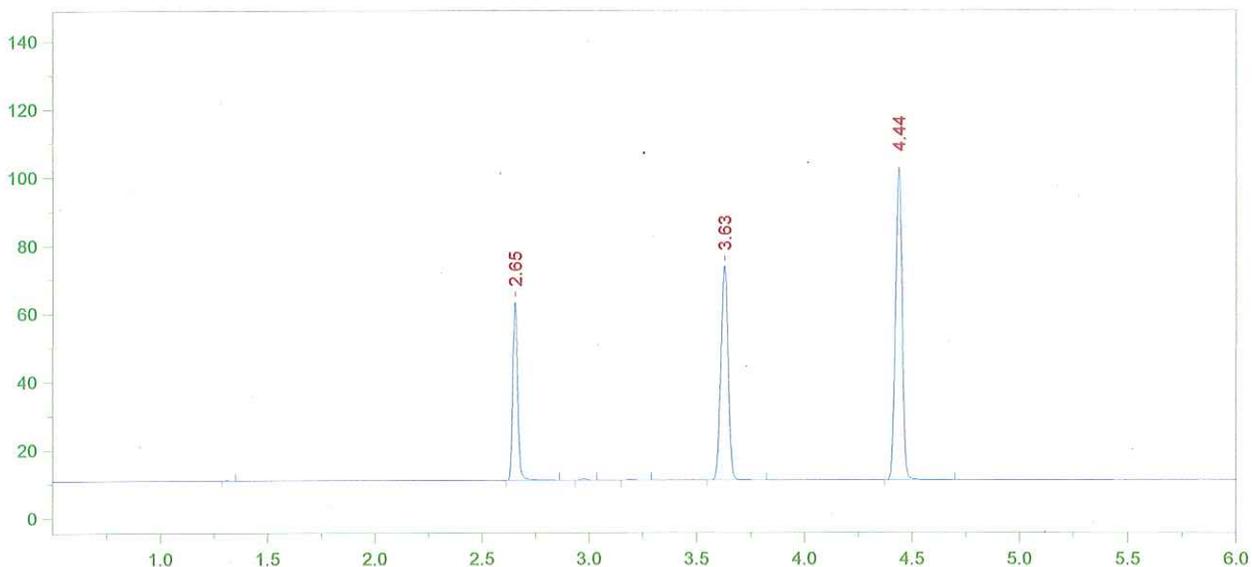
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	82840	0.0782
5	3.63	t-Butanol	154532	1.0117
6	4.44	n-Propanol	200442	1.0000

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0011.RAW  
 Sample Name = CCS  
 Date Taken = 5/20/2015 1:09:33 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 1:09:39 PM  
 VFL Headspace Alcohol  
 Calibration Version = 8

Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	81626	0.0790
5	3.63	t-Butanol	145907	0.9795
6	4.44	n-Propanol	195491	1.0000

Arg vials 10 + 11 = 0.078

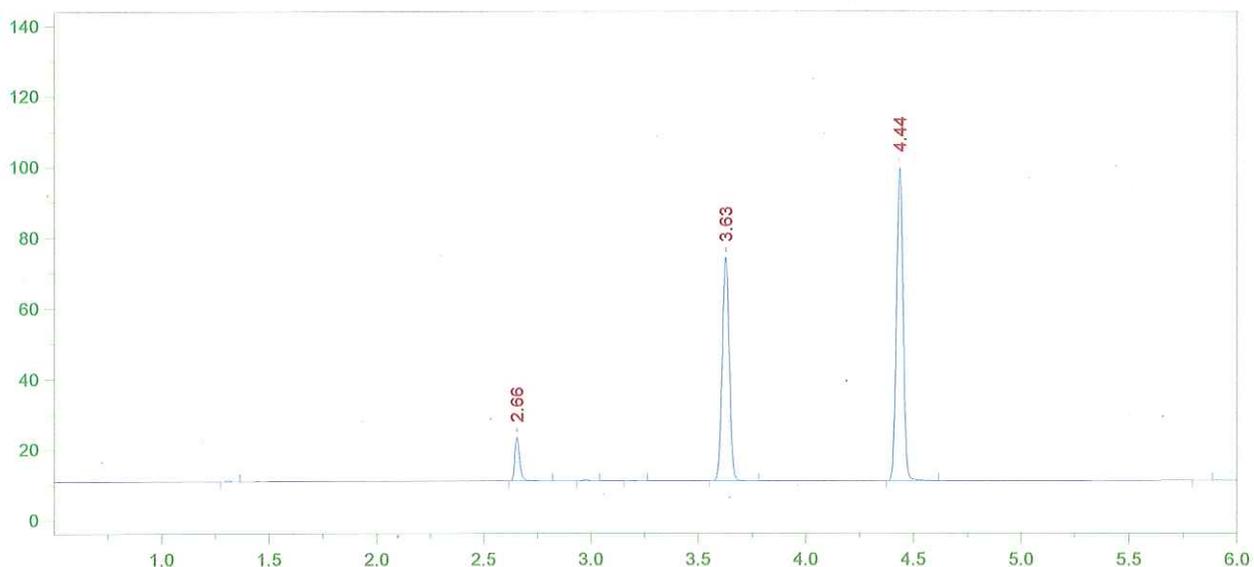
5/21/15 AB

FN 08101401

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0016.RAW  
 Sample Name = FN08101401  
 Date Taken = 5/20/2015 2:30:23 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 2:30:28 PM

VFL Headspace Alcohol

Calibration Version = 8

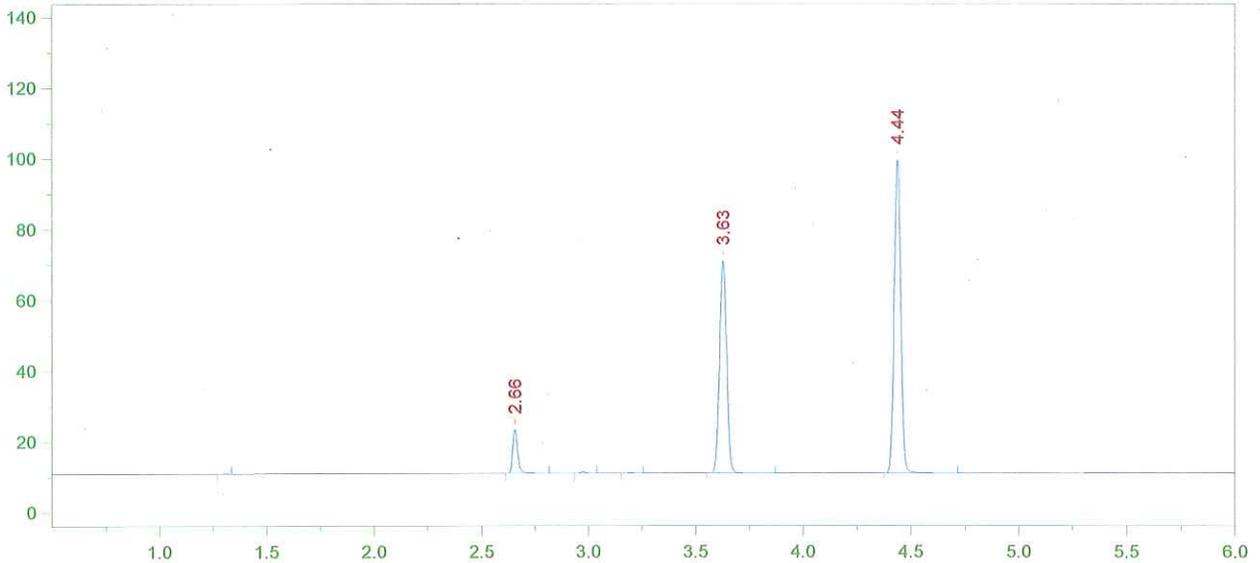
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.66	Ethanol	19942	0.0200
5	3.63	t-Butanol	147273	1.0235
6	4.44	n-Propanol	188825	1.0000

FN08101401

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AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0017.RAW  
 Sample Name = FN08101401  
 Date Taken = 5/20/2015 2:45:17 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 2:45:26 PM

VFL Headspace Alcohol

Calibration Version = 8

Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.66	Ethanol	20054	0.0201
5	3.63	t-Butanol	139530	0.9707
6	4.44	n-Propanol	188642	1.0000

Average vals 16 + 17 = 0.020

target value = 0.020

100% recovery

Cerilliant 0.02 calibration standard

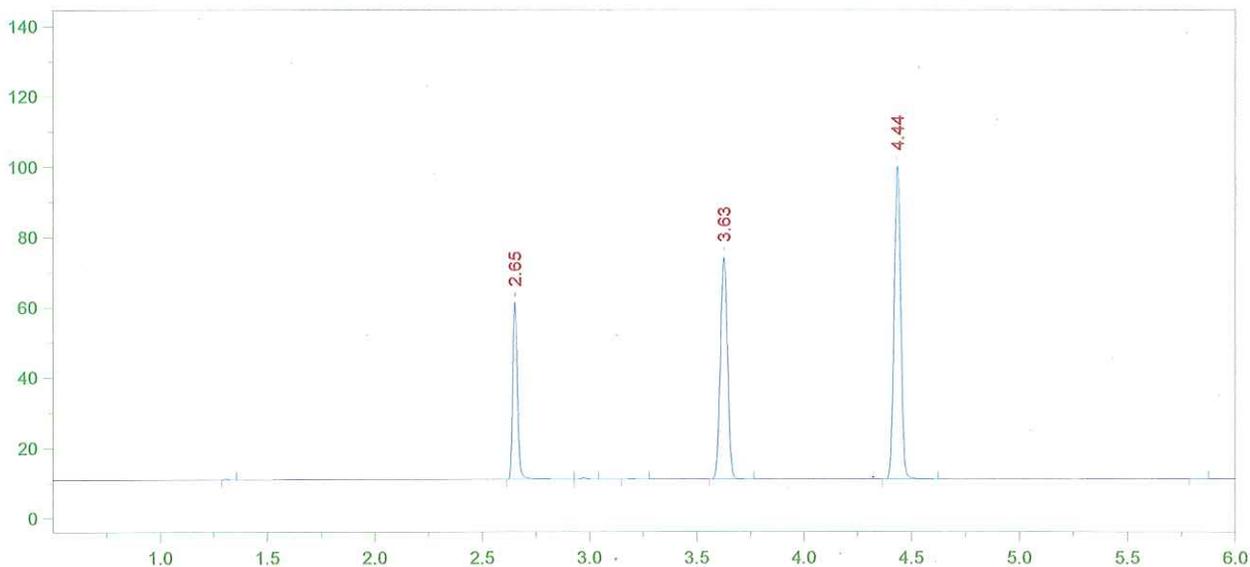
AB 5/20/15

FN08101401

15

AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0018.RAW  
 Sample Name = CCS  
 Date Taken = 5/20/2015 3:01:45 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 3:01:51 PM

VFL Headspace Alcohol

Calibration Version = 8

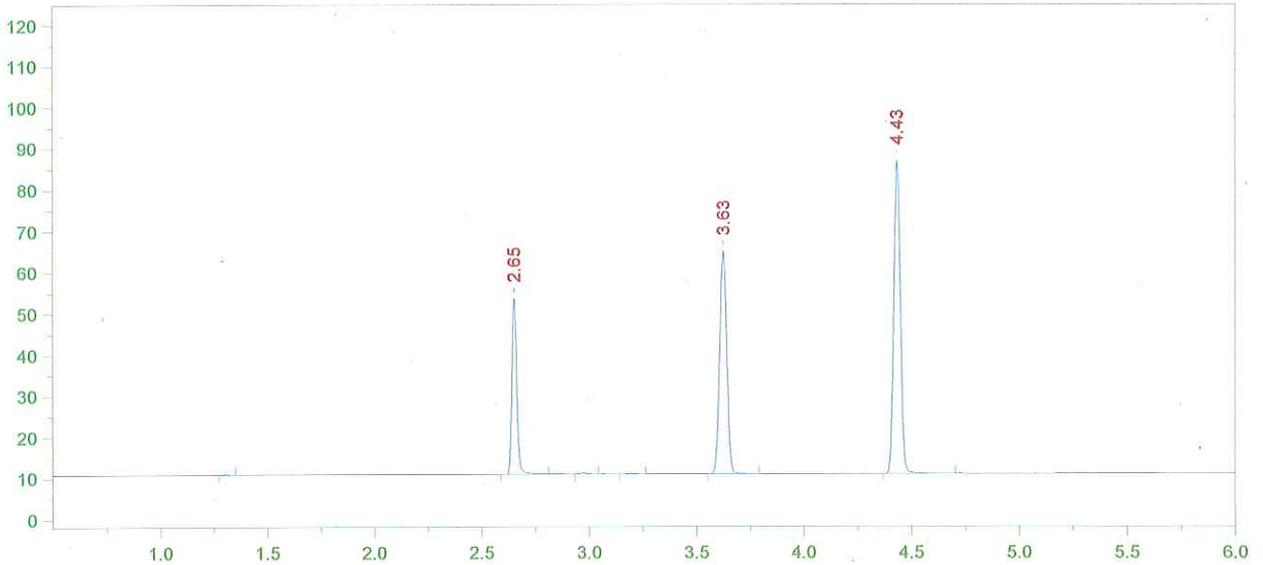
Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	78748	0.0787
5	3.63	t-Butanol	146289	1.0146
6	4.44	n-Propanol	189223	1.0000

FN 08101401

16

AB

Chrom Perfect Chromatogram Report



Format File Name = AlcoholVFL.FMT

Today's Date = 5/20/2015  
 Bound File Name = VFL05202015.0019.RAW  
 Sample Name = CCS  
 Date Taken = 5/20/2015 3:15:34 PM  
 Method file = Blood Alc temp VFL.MET  
 Calibration file = VFL05202015.CAL

Today's Time = 3:15:39 PM

VFL Headspace Alcohol

Calibration Version = 8

Peak Number	Ret Time (min)	Peak Name	Area	Amount % WT/VOL
2	2.65	Ethanol	67104	0.0786
5	3.63	t-Butanol	125324	1.0189
6	4.43	n-Propanol	161411	1.0000

*Average rats 18 + 19 = 0.078*  
*AB 5/21/15*

*AB 5/21/15*  
*F4*

*FN 08101401*

*17*

*AB*