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CHEM 1255
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Get Out of My Headspace – Determining the Blood Alcohol Content of an Unknown Using HSGC-FID

I submit this lab report as an original document. I assert that all ideas and discussion of data contained herein is my own work, unless otherwise referenced.

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Introduction

HSGC-FID is useful for the determination of volatile substances present in a sample, in contrast to regular GC, which focuses on the liquid state of the sample. The utility of only testing the vapor phase of a sample allows for an easier separation of the nonvolatile materials present in the sample. Because of this, HSGC is useful for testing samples of volatiles present in solutions, such as finding the amount of allyl isothiocyanate present in cowpea beans, and finding the amount of volatile chemicals found in orange oils.

This particular experiment was performed to verify the use of HSGC-FID to determine amounts of alcohol present in a sample of blood. HSGC-FID is used a lot in determining the BAC of individuals to determine whether or not they were driving above or below the BAC limit. In this lab, multiple stock solutions of varying ethanol concentration were produced and run through a HSGC-FID in order to produce data to use in a calibration curve. A sample of unknown ethanol concentration is also run through the HSGC-FID. Using the data from all of the samples, the concentration of ethanol in the unknown serum can be determined.

HSGC-FID is beneficial to use to determine BAC because of the focus on volatile substances. In reality, regular GC-FID cannot be used to determine BAC because of the danger of pumping the fluid through the instrument. Blood on its own tends to have multiple large and intricate compounds present and could potentially ruin the GC-FID. HSGC-FID only focus on the vapor phase present in the vial, which does not include the large compounds. Luckily, ethanol and other kinds of basic alcohols are both relatively volatile and low in mass. The alcohols in the vapor phase of the sample will be pumped through the HSGC-FID and show up in the complete scans, while not being polluted by any other components in blood.

Sample Calculations*

*[] denotes main calculation and {} denotes uncertainty calculation

[1]

$$Area\ Ratio = \frac{Area_{Eth}}{Area_{Pro}}$$

$$Area\ Ratio_{Standard\ 1} = \frac{2954.37\ \mathrm{V \cdot s}}{152513.55\ \mathrm{V \cdot s}} = 0.0193712$$

[2]

$$BAC_f = \frac{(Area\ Ratio - b)}{m}$$

$$BAC_{f\ Serum} = \frac{[0.526769 - (-0.04053)]}{2.894573\ \frac{\mathrm{dL}}{\mathrm{g}}} = 0.03220\ \frac{\mathrm{g}}{\mathrm{dL}}$$

[3]

$$BAC_0 = \frac{V_f}{V_0} \cdot BAC_f$$

$$BAC_{0\ Serum} = \frac{10\ \mathrm{mL}}{1\ \mathrm{mL}} \cdot 0.0322\ \frac{\mathrm{g}}{\mathrm{dL}} = 0.322\ \frac{\mathrm{g}}{\mathrm{dL}}$$

{1}

$$\delta Area\ Ratio = Area\ Ratio \sqrt{\left(\frac{\delta Area_{Eth}}{Area_{Eth}}\right)^2 + \left(\frac{\delta Area_{Pro}}{Area_{Pro}}\right)^2}$$

$$\delta Area\ Ratio_{Standard\ 1} = 0.0193792 \sqrt{\left(\frac{0.005\ \mathrm{V \cdot s}}{2954.37\ \mathrm{V \cdot s}}\right)^2 + \left(\frac{0.005\ \mathrm{V \cdot s}}{152513.55\ \mathrm{V \cdot s}}\right)^2} = 3 \cdot 10^{-8}$$

{2}

$$\delta BAC_f = BAC_f \sqrt{\left(\frac{\sqrt{\delta Area\ Ratio^2 + \delta b^2}}{Area\ Ratio - b}\right)^2 + \left(\frac{\delta m}{m}\right)^2}$$

$$\delta BAC_{f\ Serum} = 0.0322\ \frac{\text{g}}{\text{dL}} \sqrt{\left(\frac{\sqrt{(4 \cdot 10^{-8})^2 + 0.02^2}}{0.0526769 - (-0.04053)}\right)^2 + \left(\frac{0.07\ \frac{\text{dL}}{\text{g}}}{2.894573\ \frac{\text{dL}}{\text{g}}}\right)^2} = 0.005\ \frac{\text{g}}{\text{dL}}$$

{3}

$$\delta BAC_0 = BAC_0 \sqrt{\left(\frac{\delta BAC_f}{BAC_f}\right)^2 + \left(\frac{\delta V_f}{V_f}\right)^2 + \left(\frac{\delta V_0}{V_0}\right)^2}$$

$$\delta BAC_{0\ Serum} = 0.322\ \frac{\text{g}}{\text{dL}} \sqrt{\left(\frac{0.005\ \frac{\text{g}}{\text{dL}}}{0.0322\ \frac{\text{g}}{\text{dL}}}\right)^2 + \left(\frac{0\ \text{mL}}{10\ \text{mL}}\right)^2 + \left(\frac{0\ \text{mL}}{1\ \text{mL}}\right)^2} = 0.05\ \frac{\text{g}}{\text{dL}}$$

{4}

$$\delta m_{N\ Cigarette} = m_{N\ Cigarette} \sqrt{\left(\frac{\delta m_{N\ Sample}}{m_{N\ Sample}}\right)^2 + \left(\frac{\delta m_{Sample}}{m_{Sample}}\right)^2 + \left(\frac{\delta m_{Cigarette}}{m_{Cigarette}}\right)^2}$$

$$\delta m_N = 15\ \text{mg} \sqrt{\left(\frac{0.3\ \text{mg}}{1.02\ \text{mg}}\right)^2 + \left(\frac{0.1\ \text{mg}}{52\ \text{mg}}\right)^2 + \left(\frac{0.1\ \text{mg}}{787\ \text{mg}}\right)^2} = 4\ \text{mg}$$

Discussion

1.

Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]
1	0.145	11.49	25.45
2	0.384	28.07	41.95
3	0.672	13.23	29.98
4	0.772	23.22	27.56
5	0.798	31.16	32.52
6	0.987	21.53	51.85
7	1.058	114.14	106.59
8	1.143	13.01	24.77
9	1.177	20.13	25.73
10	1.282	64.63	25.56
11	1.393	16.82	25.07
12	1.542	18.07	28.05
13	1.766	2954.37	1046.68
14	2.029	10.90	18.36
15	2.082	33.19	32.64
16	2.244	29.77	32.70
17	2.379	23.63	20.22
18	2.431	17.88	26.67
19	2.646	15.19	22.54
20	2.681	46.29	47.55
21	2.786	11.99	22.27
22	2.952	152513.55	48462.83
23	3.280	17.08	28.58
24	3.414	21.42	30.34
		156070.76	50236.46

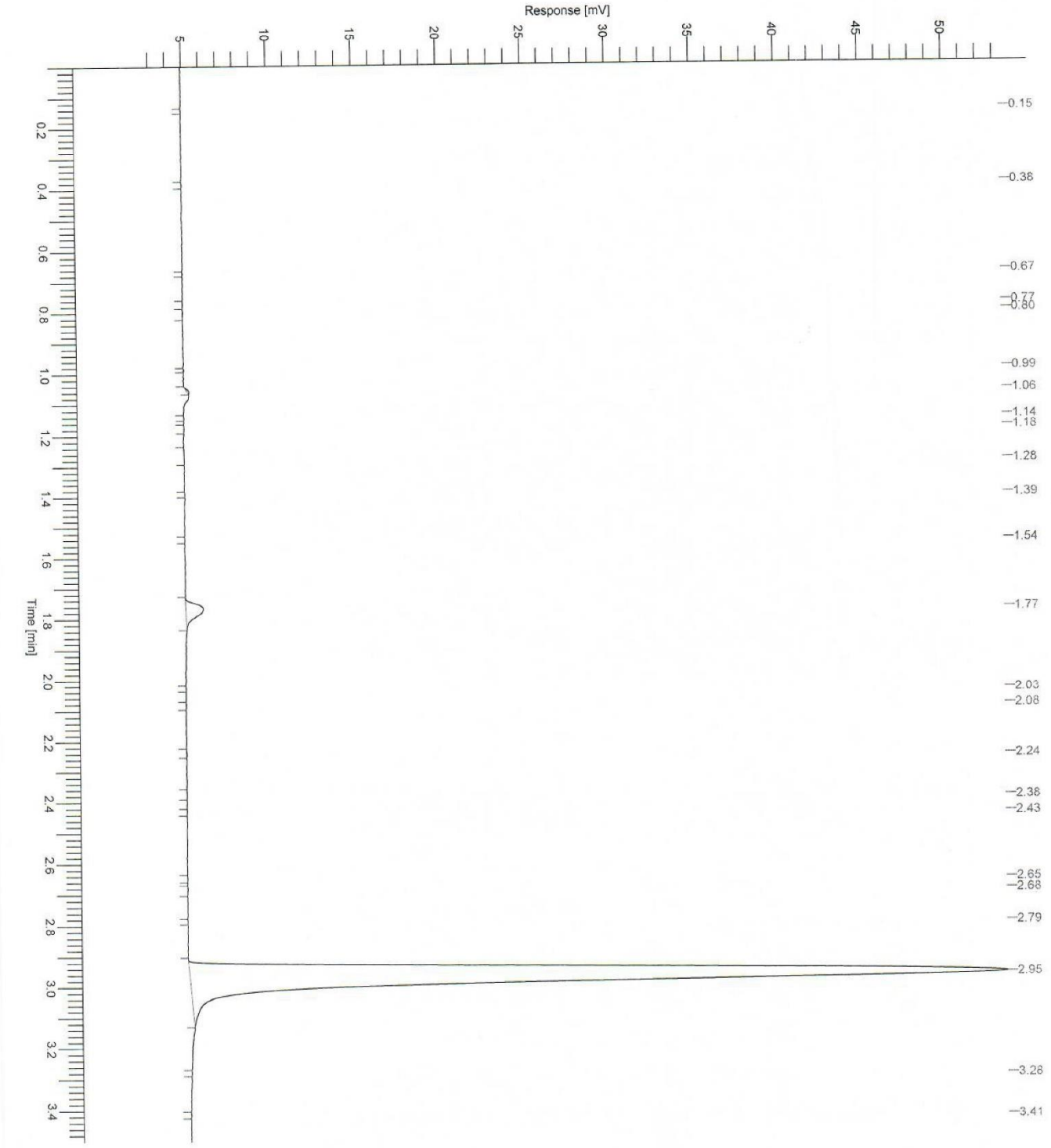
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : 0.01 Sample #: 1 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample001.raw
Date : 8/20/2020 10:32:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:28:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.45 mV High Point : 53.43 mV
Scale Factor: 1.0 Plot Offset: 2.45 mV Plot Scale: 51.0 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]
1	0.047	20.41	28.00
2	0.101	43.34	17.15
3	0.254	21.34	30.85
4	0.289	62.06	36.83
5	0.320	28.39	28.78
6	0.404	24.97	28.32
7	0.779	8.92	19.27
8	0.852	22.88	25.95
9	0.888	16.34	26.29
10	0.918	22.25	26.08
11	1.060	146.63	118.33
12	1.139	11.87	25.28
13	1.282	84.39	27.64
14	1.640	11.93	21.38
15	1.760	15289.54	5406.79
16	1.975	19.24	24.41
17	2.296	21.19	28.00
18	2.315	13.78	23.44
19	2.412	12.04	23.53
20	2.953	145915.24	46077.61
		161796.73	52043.92

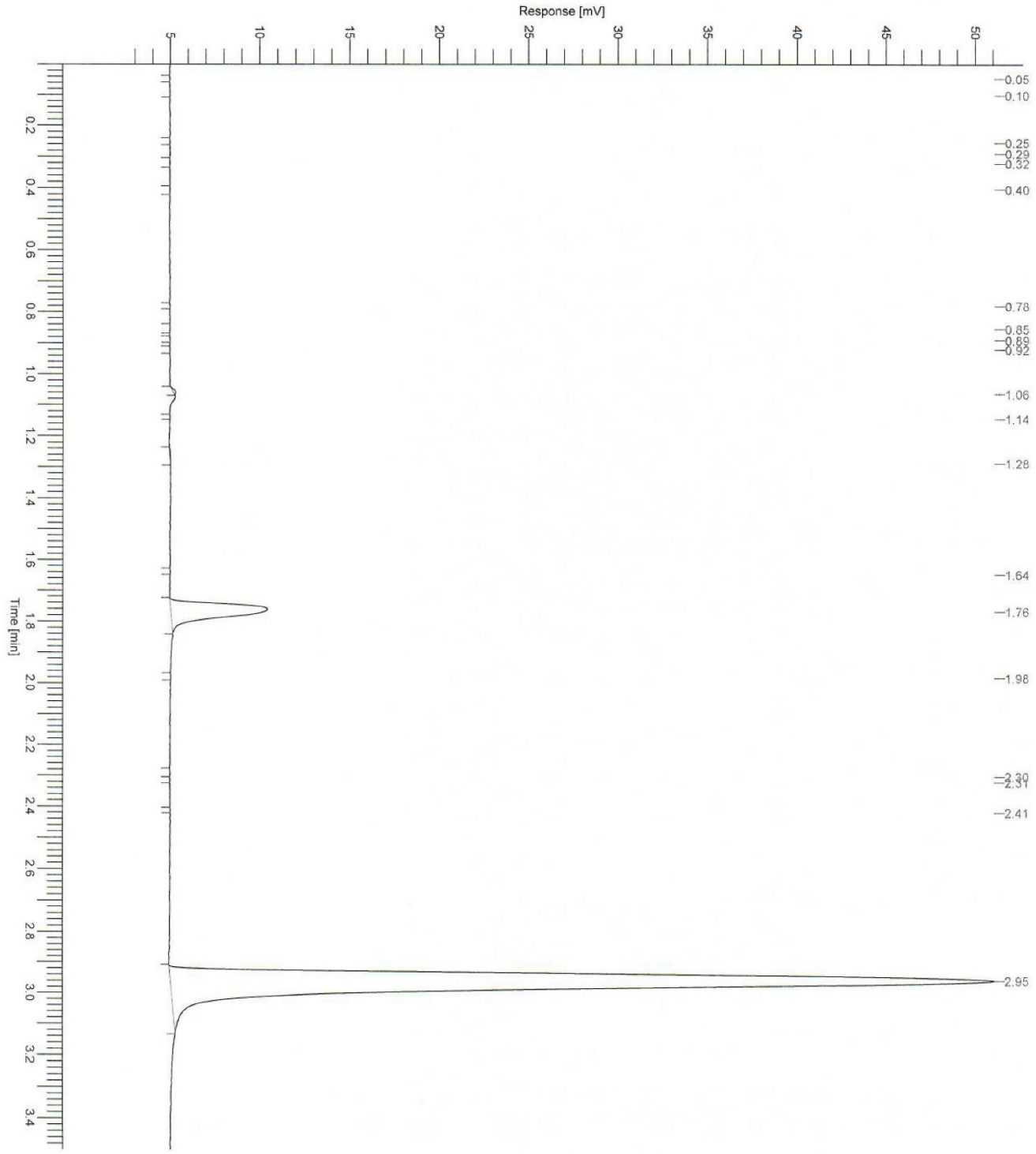
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : .05 Sample #: 2 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample005.raw
Date : 8/20/2020 10:37:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:33:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.61 mV High Point : 51.09 mV
Scale Factor: 1.0 Plot Offset: 2.61 mV Plot Scale: 48.5 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]	
1	0.076	14.69	25.45	
2	0.135	26.83	24.50	
3	0.355	23.76	26.65	
4	0.713	38.37	33.04	
5	0.968	12.61	29.45	
6	0.987	20.94	26.16	
7	1.063	820.32	313.35	
8	1.300	147.48	36.00	
9	1.322	24.58	21.90	
10	1.362	12.02	23.99	
11	1.407	23.49	35.25	
12	1.552	19.45	24.89	
13	1.631	81.09	39.92	
14	1.664	16.68	25.68	
15	1.758	26953.72	9524.78	-
16	2.885	12.55	27.28	
17	2.953	144792.95	45551.98	-
		173041.52	55790.24	

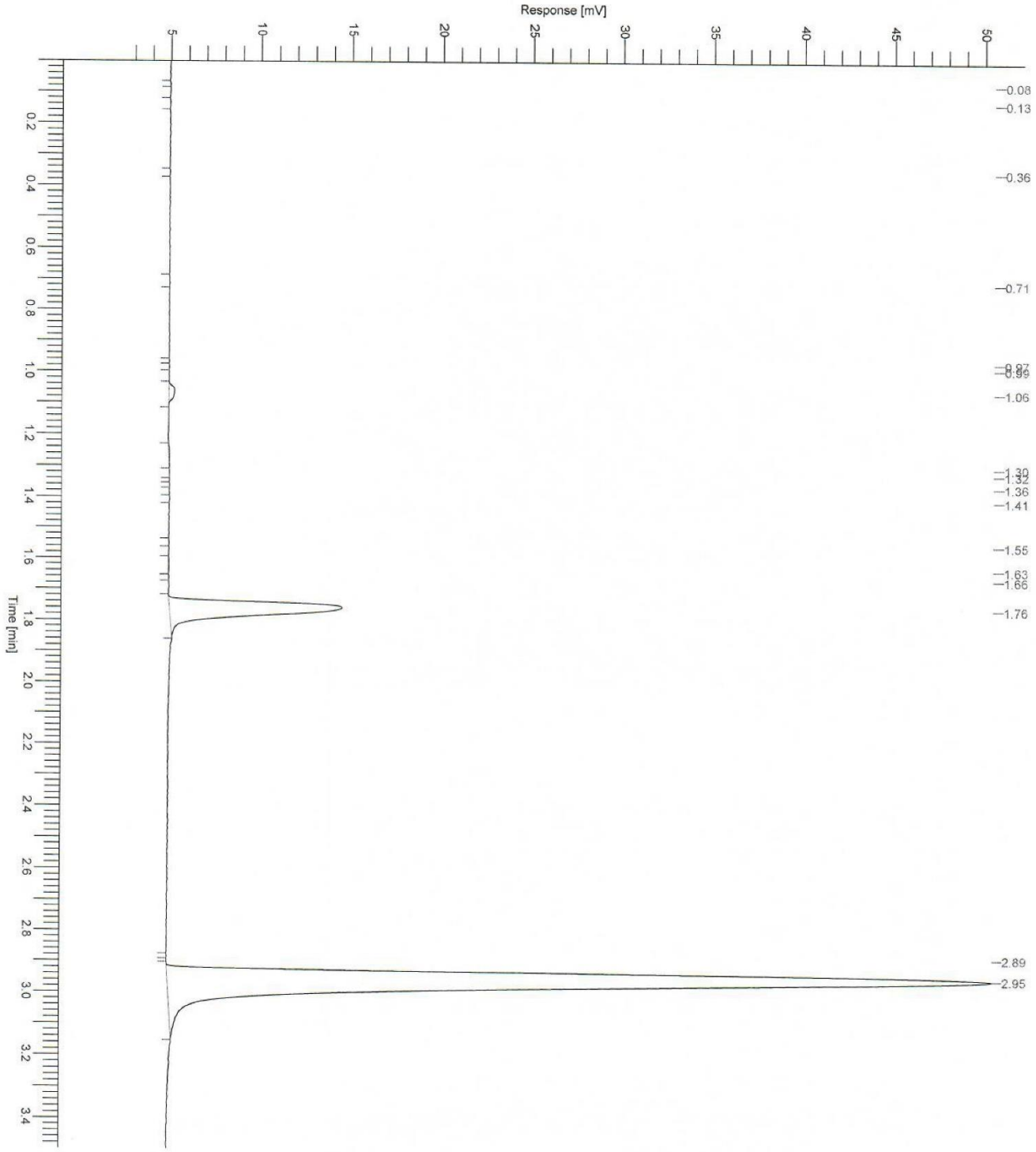
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : .08 Sample #: 3 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample008.raw
Date : 8/20/2020 10:42:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:38:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.61 mV High Point : 50.55 mV
Scale Factor: 1.0 Plot Offset: 2.61 mV Plot Scale: 47.9 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]
1	0.036	40.77	35.02
2	0.235	15.70	23.32
3	0.285	12.06	19.75
4	0.417	31.46	20.58
5	0.473	24.57	32.97
6	0.506	22.75	31.03
7	0.667	16.62	33.23
8	0.703	22.88	30.53
9	0.742	29.48	31.57
10	0.862	22.23	27.81
11	1.064	814.12	323.50
12	1.206	12.11	23.71
13	1.268	67.98	44.15
14	1.292	46.21	46.36
15	1.321	19.06	21.18
16	1.378	38.96	36.06
17	1.510	111.89	57.79
18	1.649	21.93	29.09
19	1.757	35508.56	12489.56
20	2.206	14.11	20.96
21	2.403	19.47	25.13
22	2.522	16.57	17.97
23	2.665	12.95	17.63
24	2.954	148617.28	46860.11
		185559.71	60299.05

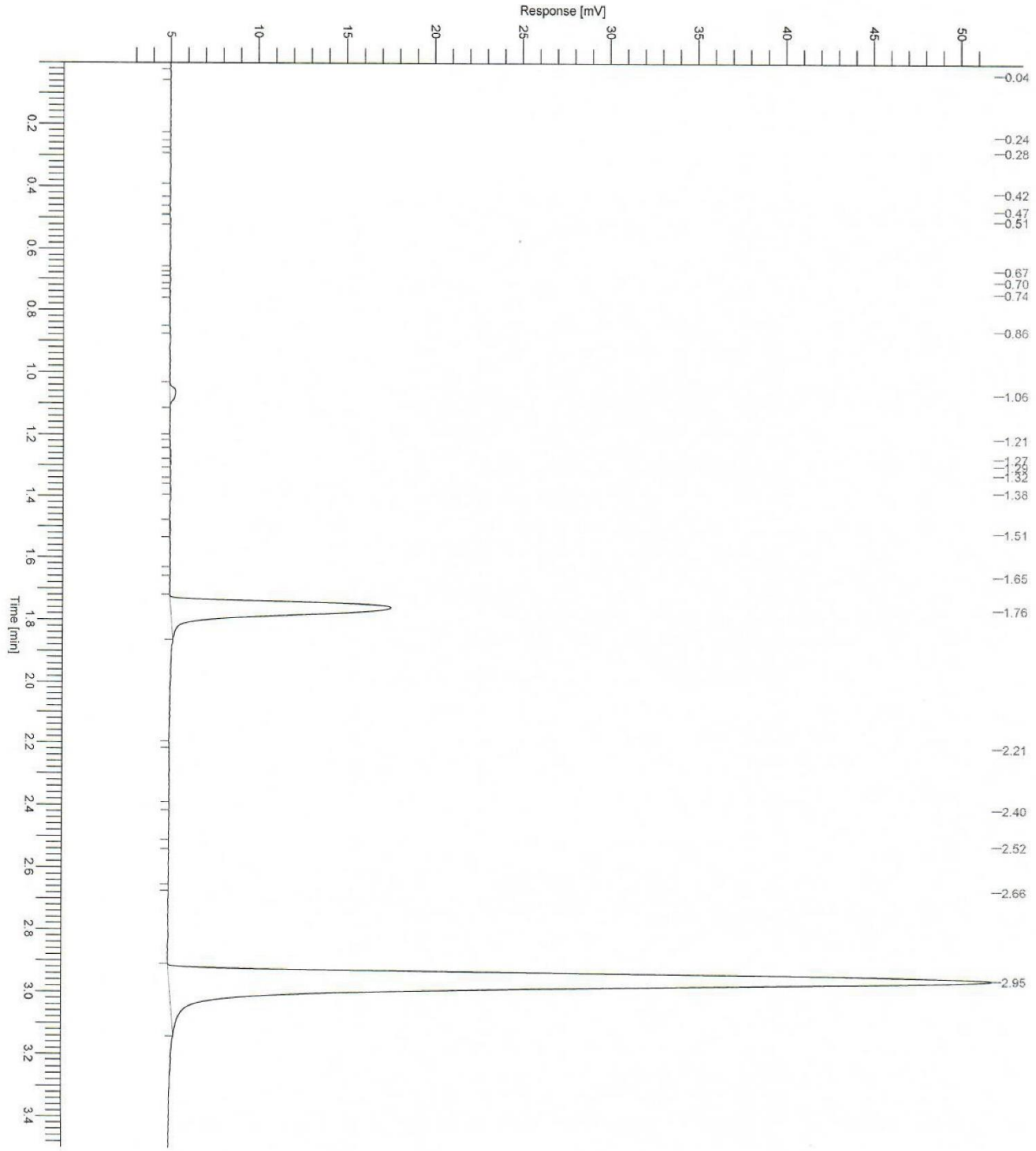
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : .1 Sample #: 4 Page 1 of 1
FileName : c:\tcdata\data\verick_fall2020\sample01.raw
Date : 8/20/2020 10:47:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:43:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.60 mV High Point : 51.89 mV
Scale Factor: 1.0 Plot Offset: 2.60 mV Plot Scale: 49.3 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]
1	0.183	30.41	27.67
2	0.370	22.58	22.14
3	0.535	21.86	24.57
4	0.602	22.50	26.26
5	1.061	790.32	311.42
6	1.258	28.24	33.19
7	1.391	7049.72	2359.78
8	1.625	35.06	27.38
9	1.653	49.27	43.41
10	1.753	75276.88	26932.53
11	2.145	20.24	33.23
12	2.327	218025.09	75667.03
13	2.785	14.17	18.70
14	2.954	147769.30	46686.16
15	3.355	43.40	35.86
		449199.03	152249.35

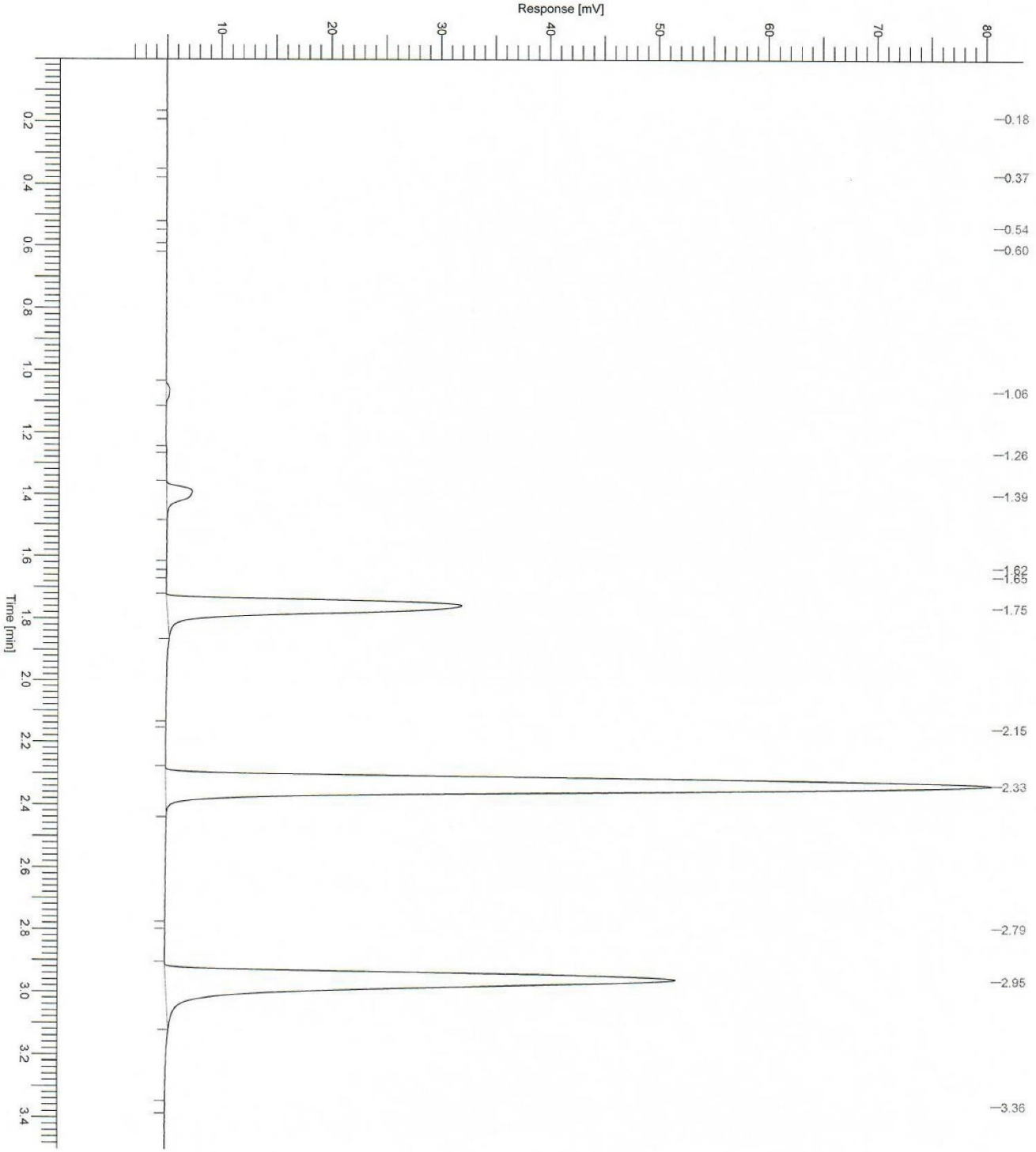
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : .2 Sample #: 5 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample02.raw
Date : 8/20/2020 10:52:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:48:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 1.14 mV High Point : 80.72 mV
Scale Factor: 1.0 Plot Offset: 1.14 mV Plot Scale: 79.6 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]
1	0.387	22.25	37.06
2	0.414	28.76	25.12
3	0.470	18.19	30.45
4	0.706	15.04	25.82
5	0.963	27.74	27.66
6	1.060	796.59	318.14
7	1.209	24.31	28.46
8	1.259	23.86	25.99
9	1.298	22.49	24.00
10	1.516	35.29	30.03
11	1.542	12.39	22.39
12	1.752	114973.74	41178.76
13	2.141	119640.30	41250.29
14	2.734	31.67	28.35
15	2.808	20.25	26.51
16	2.827	19.60	28.49
17	2.954	142185.82	44264.95
18	3.334	16.34	23.84
		377914.63	127396.31

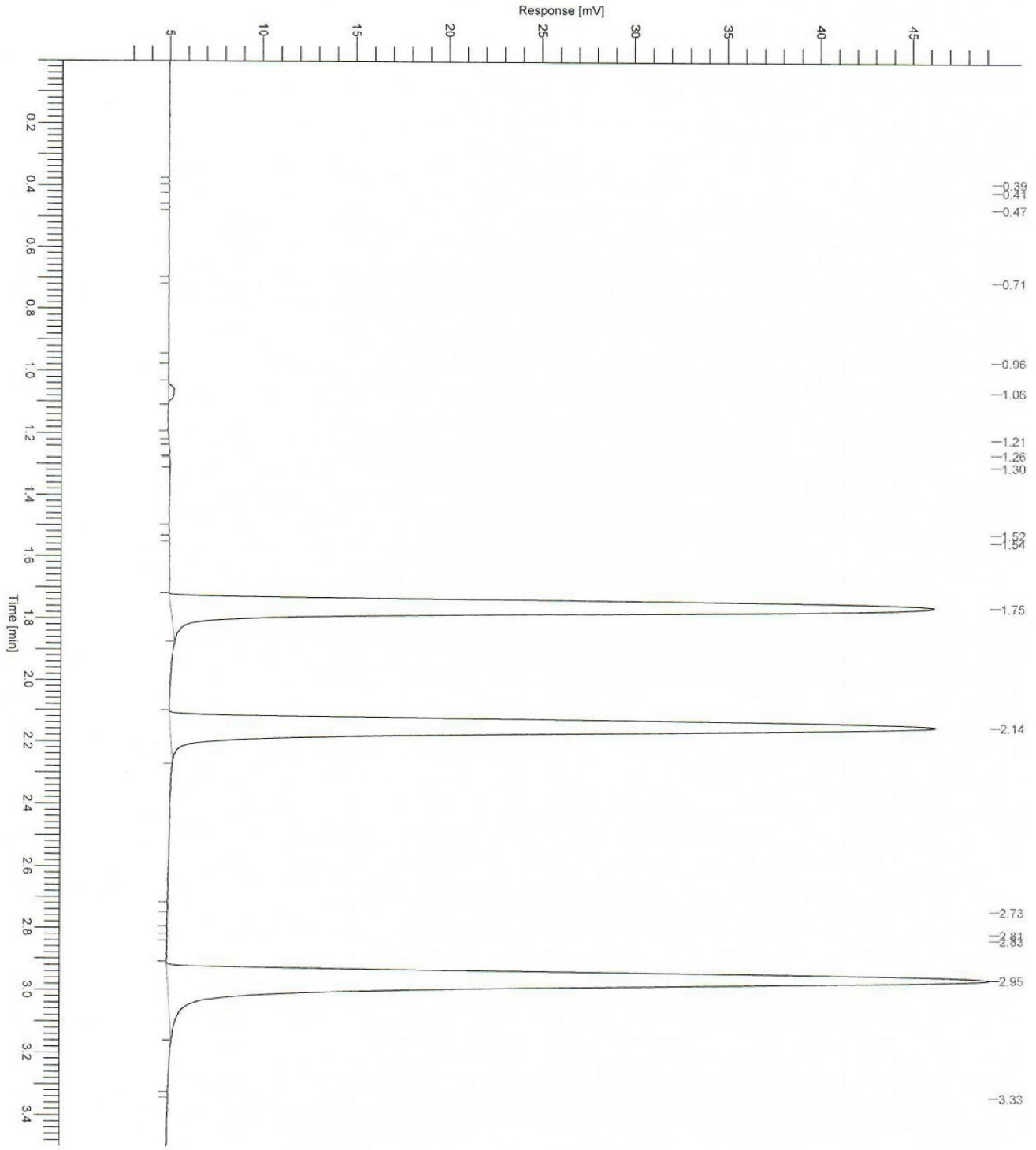
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : .3 Sample #: 6 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample03.raw
Date : 8/20/2020 10:57:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:53:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.71 mV High Point : 49.29 mV
Scale Factor: 1.0 Plot Offset: 2.71 mV Plot Scale: 46.6 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]	
1	0.074	31.51	36.31	
2	0.269	18.86	28.86	
3	0.328	41.34	33.62	
4	0.528	38.91	36.55	
5	0.639	15.81	24.29	
6	0.717	19.41	32.00	
7	0.865	14.88	22.47	
8	0.972	15.49	26.55	
9	1.060	108.15	94.79	
10	1.235	149.67	82.30	
11	1.255	101.18	74.01	
12	1.291	55.65	31.59	
13	1.385	135614.03	47684.59	
14	1.654	18.02	26.02	
15	1.751	156269.64	55618.64	-
16	2.318	13.68	22.09	
17	2.456	39.83	35.46	
18	2.483	10.81	21.84	
19	2.832	14.57	20.30	
20	2.955	136025.17	42559.14	-
21	3.423	19.25	29.84	
		428635.88	146541.26	

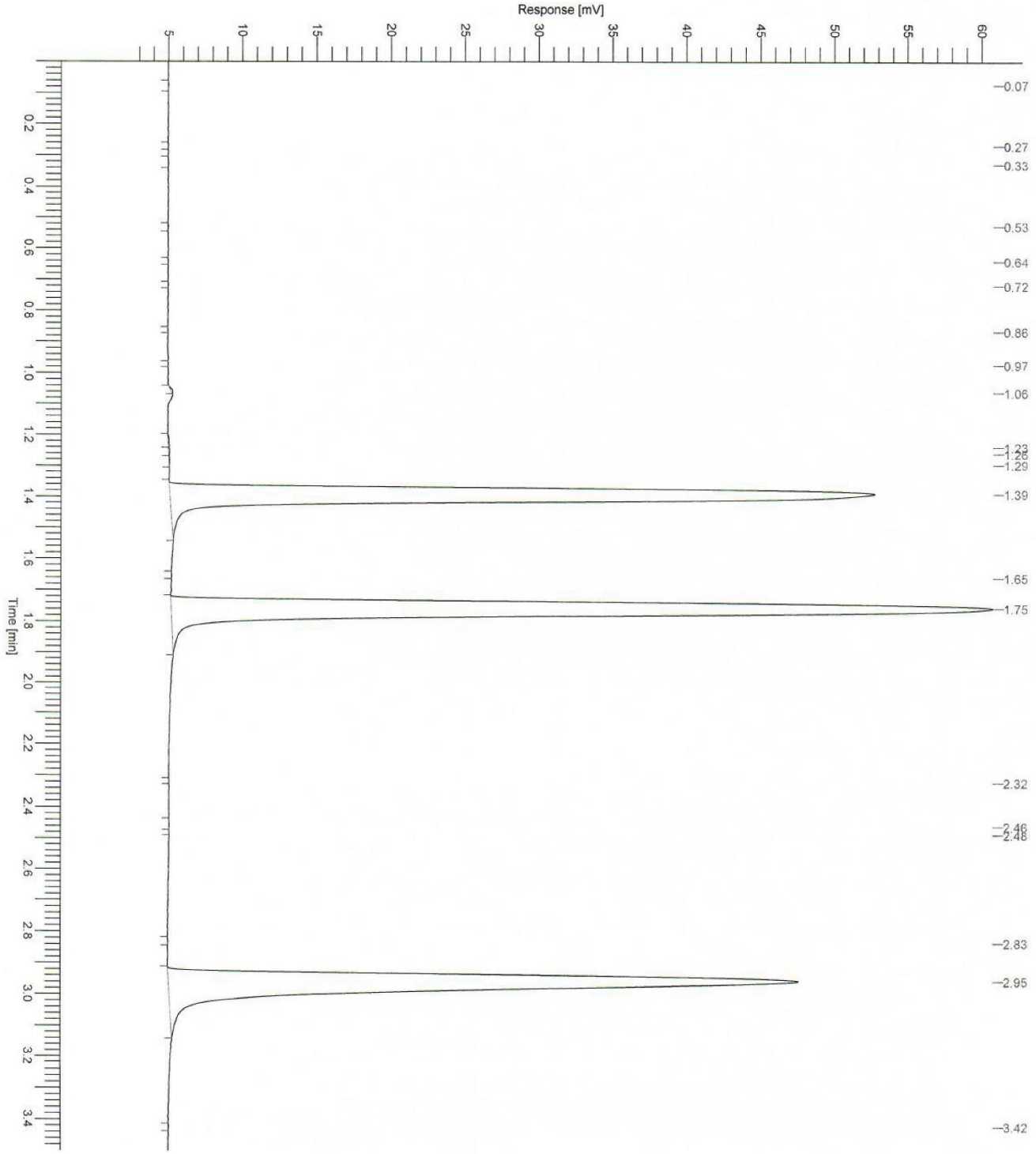
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : .4 Sample #: 7 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample04.raw
Date : 8/20/2020 11:02:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 10:58:51 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.13 mV High Point : 60.82 mV
Scale Factor: 1.0 Plot Offset: 2.13 mV Plot Scale: 58.7 mV



Blood Alcohol Content

Peak #	Time [min]	Area [uV*sec]	Height [uV]
1	0.227	16.14	29.42
2	0.248	25.43	35.02
3	0.383	29.00	29.50
4	0.486	30.05	24.12
5	0.525	19.78	35.74
6	0.588	23.30	34.35
7	0.979	31.77	26.60
8	1.031	13.82	26.03
9	1.064	825.42	328.92
10	1.209	81.10	78.01
11	1.255	14.70	21.83
12	1.323	578.25	198.61
13	1.486	18.04	20.78
14	1.554	24.19	31.90
15	1.572	24.59	33.41
16	1.658	14.23	25.58
17	1.766	7345.61	2513.87
18	2.010	30.94	19.27
19	2.052	35.60	38.61
20	2.159	32.03	26.56
21	2.249	32.33	32.05
22	2.350	104.36	46.40
23	2.821	22.66	35.10
24	2.955	139446.47	43617.23
		148819.82	47308.91

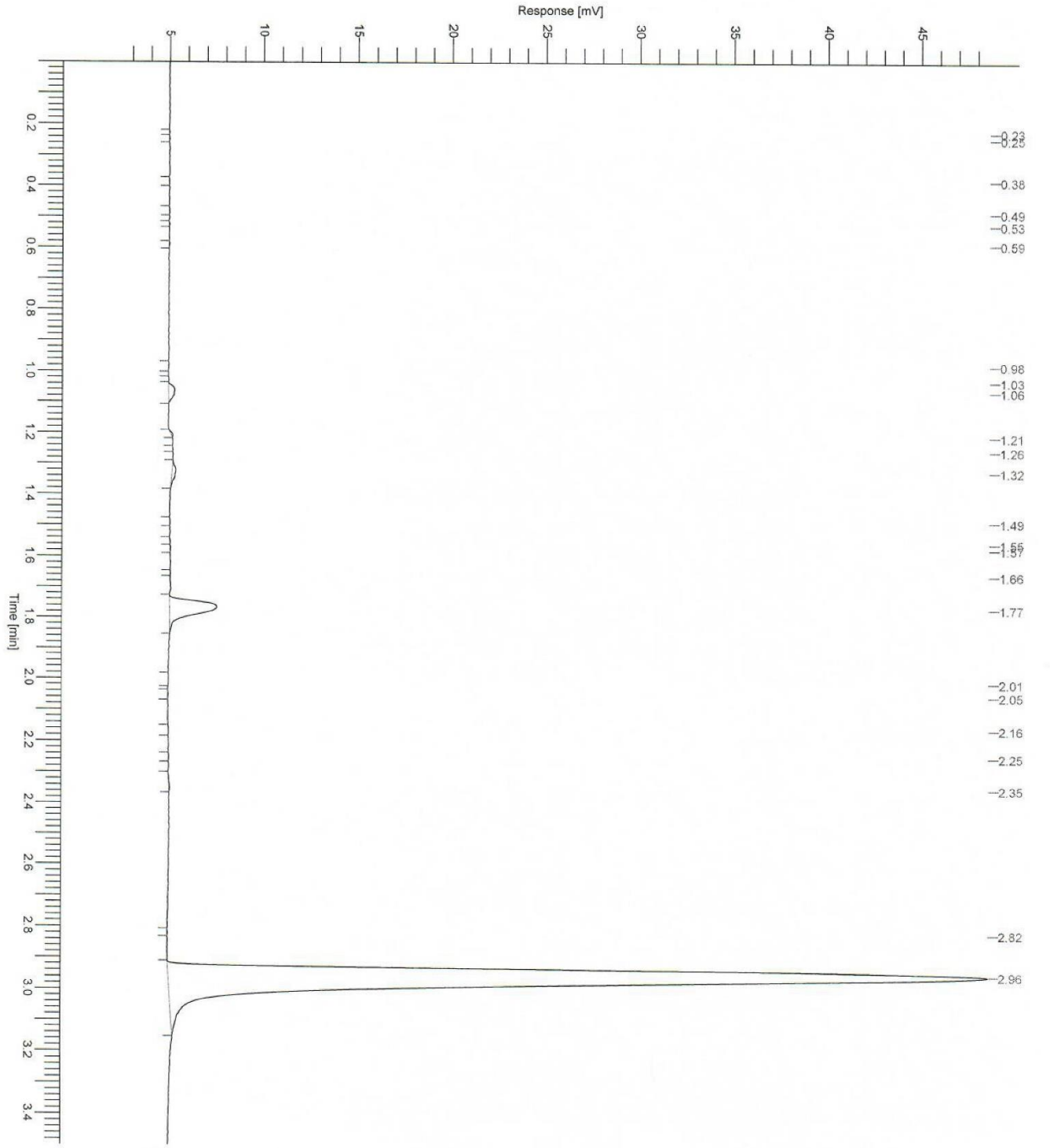
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

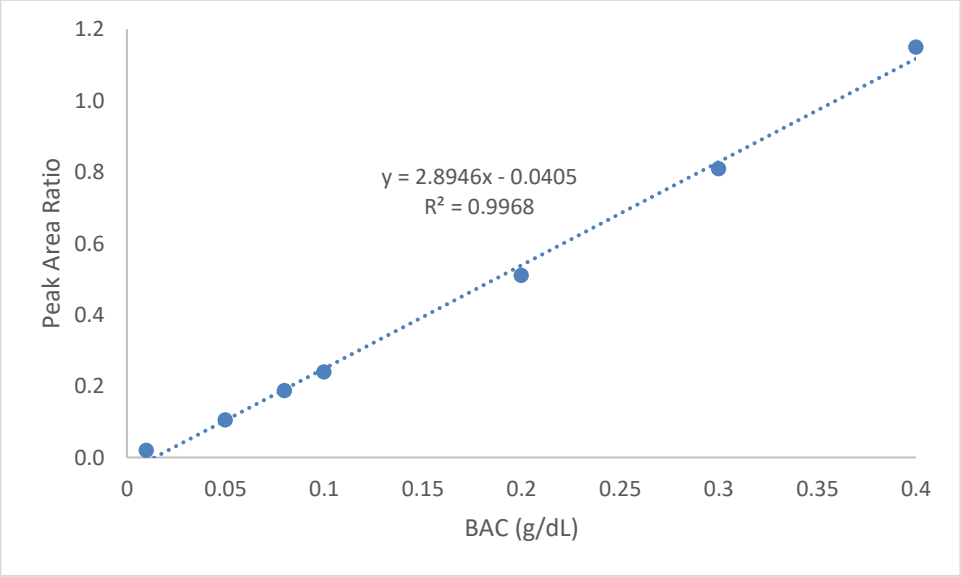
Chromatogram

Sample Name : Serum Sample #: 8 Page 1 of 1
FileName : c:\tcdata\data\rerick_fall2020\sample_unknown.raw
Date : 8/20/2020 11:07:30 AM
Method : michelle.mth Time of Injection: 8/20/2020 11:03:50 AM
Start Time : 0.00 min End Time : 3.50 min Low Point : 2.75 mV High Point : 48.65 mV
Scale Factor: 1.0 Plot Offset: 2.75 mV Plot Scale: 45.9 mV



2.

Sample #	BAC (g/dL)	Peak Area of Ethanol (V*s)	Peak Area of Propanol (V*s)	Peak Area Ratio [1] {1}
1	0.01 ± 0.005	2954.37 ± 0.005	152513.55 ± 0.005	$0.0193712 \pm 3\times10^{-8}$
2	0.05 ± 0.005	15289.54 ± 0.005	145915.24 ± 0.005	$0.1047837 \pm 3\times10^{-8}$
3	0.08 ± 0.005	26953.72 ± 0.005	144792.95 ± 0.005	$0.1861535 \pm 4\times10^{-8}$
4	0.10 ± 0.005	35508.56 ± 0.005	148617.28 ± 0.005	$0.2389262 \pm 3\times10^{-8}$
5	0.20 ± 0.005	75276.88 ± 0.005	147769.30 ± 0.005	$0.5094216 \pm 4\times10^{-8}$
6	0.30 ± 0.005	114973.74 ± 0.005	142185.82 ± 0.005	$0.80861608 \pm 5\times10^{-8}$
7	0.40 ± 0.005	156269.64 ± 0.005	136025.17 ± 0.005	$1.1488289 \pm 6\times10^{-8}$



Number Type	Slope	Y-Intercept
Value	2.8946	-0.0405
Uncertainty*	0.07	0.02

*Uncertainty values found through LINEST formula in excel.

3.

Sample	Peak Area of Ethanol (V*s)	Peak Area of Isopropanol (V*s)	Peak Area Ratio [1] {1}	BAC_2 (g/dL) [2] {2}
Serum	7345.61 ± 0.005	139446.47 ± 0.005	$0.0526769 \pm 4\times10^{-8}$	0.0322 ± 0.005

4.

Sample	BAC_2 (g/dL) [2] {2}	V_2 (mL)	V_1 (mL)	BAC_1 (g/dL) [3] {3}
Serum	0.0322 ± 0.005	10	1	0.322 ± 0.05

The data for the BAC received from the unknown serum came out to be 0.322 g/dL BAC, which is significantly over the legal limit of 0.08 g/dL BAC. The driver, in this case, has broken the law.

5. The unknown serum shows no significant amount of other volatile substances that could possibly be present. The other volatiles, that were inserted into different standard solutions, can possibly appear in blood through

the consumption of alcohol. The reactions that the alcohol can cause in the body can lead to the formation of these volatiles.

6. N-propanol is a good internal standard to use in this case because n-propanol is not found naturally in blood. Any of the other substances to show up on the HSGC-FID readings are present in blood samples. If a sample that was found in blood was used as an internal standard, the data would be skewed in different ways and inconclusive.

7. If the serum isn't diluted, the peaks would be much more extreme, and decrease the resolution of samples with lower amounts.

8. In contrast to regular GC-FID, HSGC-FID analyzes only the vapors present in the sample vial. If the liquid from the "blood" was injected, a lot of much heavier compounds could be picked up and potentially ruin the GC.

9. Changing the time would either stretch or squeeze the peaks along the x-axis, which could potentially make the scan harder to read.

10. The FID ignites the samples that are run through the GC and produce water vapor, and the water vapor is ignored by the GC.