Validation Summary for Alcohol Analysis by Headspace GC-FID/MS

This document describes the validation of a procedure to quantitate ethanol in whole blood samples. Blood samples were diluted with an internal standard solution and analyzed by Headspace Gas Chromatography with simultaneous flame ionization (FID) and mass spectrometry detection (MS). An Agilent G1888 Headspace Sampler connected to an Agilent 7890A gas chromatograph (GC) with a 5975C Mass Spectrometer (MS), designated as HS-GCMS, was used for all validation experiments. The flame ionization detector (FID) was utilized for quantitation of ethyl alcohol and the mass spectrometer (MS) was used for qualitative confirmation. Other volatile compounds may be identified qualitatively by this procedure following complete validation for that compound. Please see SOP for method details. This validation summary includes the evaluation of:

1. Headspace oven thermostat time
2. Thermostat stability
3. Sensitivity
4. Linearity
5. Matrix effects
6. Carryover
7. Reproducibility
8. Drift/Bias
9. Specificity
10. Reportable range
11. Case comparison (Crossover study)

Experiments:

Experiment #1:

4/23/10	Headspace oven thermostat time
Analyst:	Nick Tiscione (NBT)

Thirty 0.100 g% ethanol standards (Cerilliant Lot # FN060707-02) were prepared by diluting with an n-propanol internal standard solution (ISTD 448) and analyzed using an incremented headspace oven thermostat time. The samples were analyzed on 4/23/10 to evaluate thermostat times of 1 to 30 minutes incremented by 1 minute for each successive sample.

Experiment #2:

5/24-5/26/10	Linearity, Matrix effects, Sensitivity, Carryover, and Thermostat stability
Analyst:	NBT

Ethanol standards were prepared and analyzed in three matrices (deionized water, human urine, and human whole blood) using the following procedure:

1. Prepare a 2.0 g% stock solution for each matrix by adding 667uL (Pipette SN 300773) of ~95% ethanol (39208/EM) to a 25 mL volumetric flask and bringing to volume with deionized (DI)

water (096104/Fisher), Preservative-free blank urine (3328/UTAK), and blank whole blood (3893/UTAK).

2. Prepare standards using the 2.0 g% stock solution by adding the following volumes to a 10 mL volumetric flask and bringing to volume with the appropriate matrix. The standards were transferred to 10 mL gray stoppered vials containing sodium fluoride and potassium oxalate (614303/Tri-Tech, Inc.) and stored under refrigeration.

Standard Concentration (g%)	Volume of 2g% stock (uL)	Pipette Serial Number
1.000	5000	203151
0.500	2500	203151
0.300	1500	203151
0.080	400	300773
0.025	125	198425
0.010	50	185045
0.005	25	185045

3. Analyze standards for all 3 matrices including a matrix matched internal standard blank sampled and run after the 1.0 g% standard for each matrix as well as typical calibrators utilized for casework. (30 samples)

Experiment #3:
6/1-6/4/10 Within-run and Between-run Reproducibility
Analysts: Tate Yeatman (DTY), Xiaoqin Shan (XS), Ilene Alford (IKA), and NBT

Each analyst (DTY, XS, IKA, and NBT) analyzed 10 replicates of the 0.025, 0.080, and 0.300 g% prepared whole blood standards along with the typical calibrators utilized for casework on four separate days. Each analyst performed the complete procedure including preparation of replicates listed above.

Experiment #4:
4/26/10, 4/30/10, 5/6/10 Within-run Reproducibility and Drift/Bias
Analysts: NBT and XS

Sixty-five replicates each of aqueous standards at 0.025 and 0.300 g% (by NBT) and a whole blood volatiles control at 0.075 g% (by XS) were analyzed on three separate days along with an internal standard blank and 4 calibrator standards.

Experiment #5:
2/1/10, 4/16/10 Specificity
Analyst: NBT

1. The following volatile solutions were prepared on 2/1/10 by adding the specified volume indicated below to 10mL of deionized water (084887/Fisher).

Volatile	Volume (uL)	Lot / Manuf.
Ethyl acetate	5	48165 / EMD
Chloroform	5	47106 / EMD
Methylene Chloride	5	CV121 / B&J
Acetonitrile	5	47156 / EMD
Acetaldehyde	2	35139 / EM
Hexanes	10	G31E29 / JTBaker
Heptane	5	47248 / EMD
Toluene	5	46059 / EMD
n-Butyl Acetate	5	47249739 / EMD
1-Chlorobutane	5	47312 / EMD
Pentane	5	32195 / EM
Iso Amyl Alcohol	5	8020 / EM

2. Common volatile inhalation anesthetics were prepared previously at the following concentrations.

Anesthetic	Concentration (% v/v)	ID
Isoflurane	0.025	CON 1003
Sevoflurane	0.10	CON 1009
Desflurane	0.10	CON 1008

3. 1,1-Difluoroethane was prepared at 0.27 mg/mL in deionized water (CON 1307).

4. 100 uL of each volatile solution prepared above was diluted with 1 mL of n-propanol internal standard (ISTD 447). A whole blood volatiles control containing methanol, ethanol, isopropanol, and acetone (0904148/Cliniqa) was also prepared using the same protocol.

5. The samples were then analyzed with the method.

Experiment #6:

4/15-5/28/10 Case comparison (Crossover study)

Analysts: NBT, IKA, DTY, XS

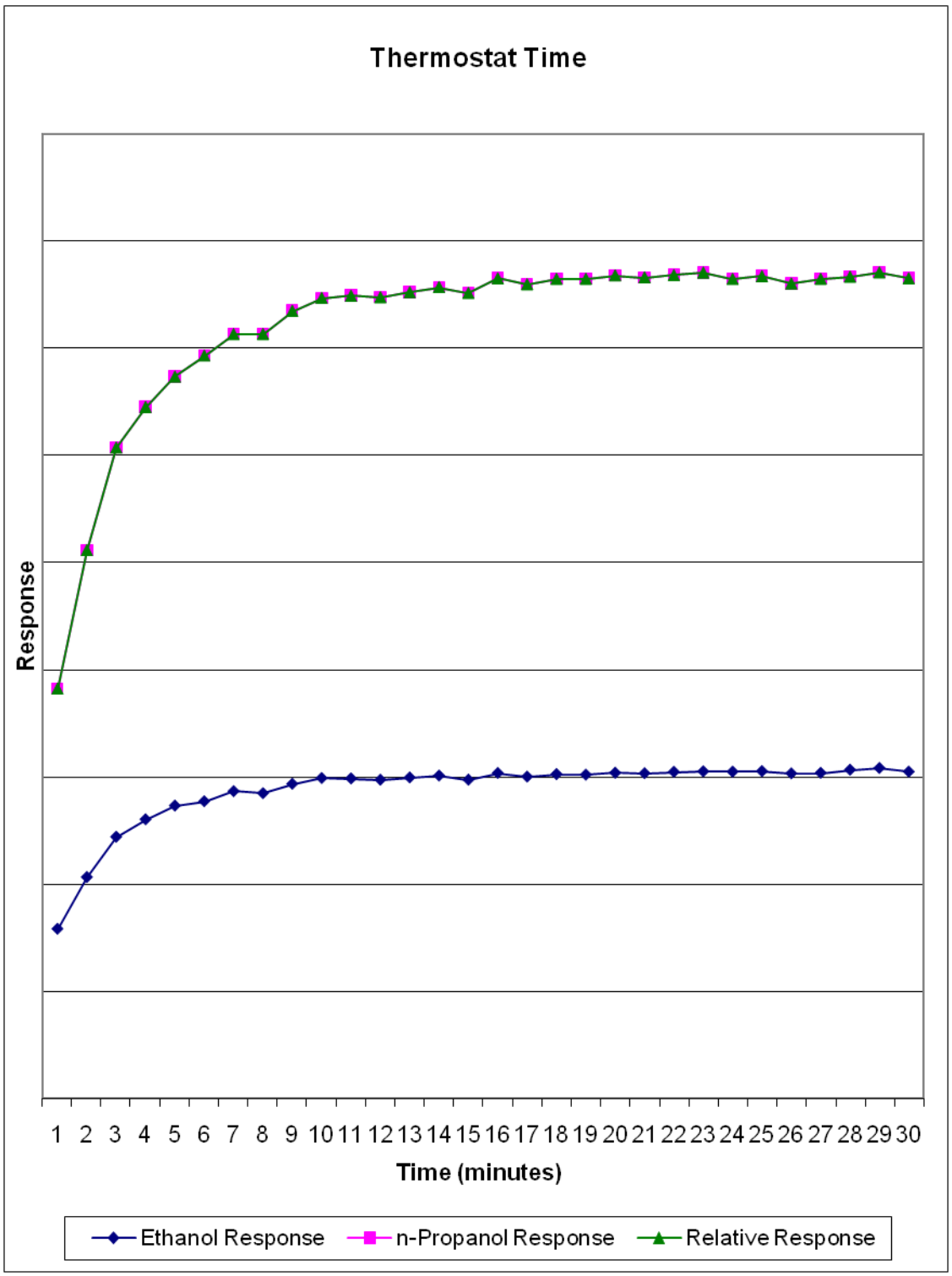
Case samples and proficiency samples from two cycles of the FDLE Alcohol Testing Program (10-Q1 and 10-Q2) that were analyzed with the currently approved instrument and SOP (PE-2 and 785.734.3, respectively) for the quantitation of ethanol were reanalyzed on the HS-GCMS. A total of 81 samples were compared; 59 positive for ethanol and 22 negative for ethanol.

Results

1. Headspace Oven Thermostat Time

Thermostating oven temperature was set at 50°C to maximize partitioning of the volatiles into the headspace while not causing degredation of ethanol to acetaldehyde in whole blood specimens during equilibration (1). The equilibration time was evaluated from 1 to 30 minutes.

Thermostat Time (min)	Ethanol Response	n-Propanol Response	Relative Response
1	1582761	2239664	0.70670
2	2065909	3050699	0.67719
3	2442481	3631333	0.67261
4	2606010	3846026	0.67759
5	2733774	4002461	0.68302
6	2774576	4158224	0.66725
7	2868745	4264614	0.67269
8	2849567	4285028	0.66501
9	2937571	4413393	0.66560
10	2991502	4480321	0.66770
11	2987649	4504985	0.66319
12	2975527	4502142	0.66091
13	2997121	4527545	0.66197
14	3014351	4557902	0.66135
15	2976095	4541110	0.65537
16	3038387	4615982	0.65823
17	3004393	4594932	0.65385
18	3027667	4621920	0.65507
19	3022699	4627566	0.65319
20	3040543	4641530	0.65507
21	3036085	4627322	0.65612
22	3049169	4635479	0.65779
23	3053725	4654501	0.65608
24	3050349	4598313	0.66336
25	3055479	4618903	0.66152
26	3034543	4570744	0.66391
27	3039591	4610139	0.65933
28	3068945	4600102	0.66715
29	3084967	4624442	0.66710
30	3051502	4603193	0.66291



Conclusion

Equilibrium is reached for ethanol and the internal standard n-propanol at 10 minutes of thermostatting at 50°C. Vials should therefore be heated for at least 10 minutes to ensure equilibrium of the volatiles concentration between the liquid and headspace is reached. Twenty minutes was chosen as the set point for the method.

2. Thermostat stability

Ethanol has been shown to degrade while thermostatting whole blood samples at temperatures greater than 50°C (1-2). To prevent the oxidative loss of ethanol in whole blood while thermostating for headspace analysis addition of sodium dithionite as an inhibitor (2) or temperatures less than or equal to 50°C have been recommended (1). To verify that no ethanol degradation occurs through oxidative loss during thermostatting at 50°C for 20 minutes without the use of sodium dithionite, whole blood standards were prepared and analyzed up to 1.0 g% ethyl alcohol. No detectable amounts of acetaldehyde (the product of oxidation of ethanol by oxyhemoglobin in whole blood) were present in whole blood standards up to 1.0 g%.

Conclusion

Thermostatting at 50°C for 20 minutes without the addition of sodium dithionite does not cause degradation of ethanol present in whole blood samples to acetaldehyde.

3. Sensitivity

Ethanol standards prepared in whole blood, urine, and aqueous matrices were analyzed. The quantitative results obtained were within ±22% of the target prepared concentration at 0.005 g% and within ±9% at 0.010 g%. For ethanol quantitation it has been recommended that the accuracy of all calibrators be within ±10% (3). Quantitation and confirmation are performed simultaneously by FID and MS, respectively. The signal to noise ratio (S/N) of the 0.005 g% standard was greater than 24:1 by FID and greater than 3.5:1 by MS for all matrices. Recommended S/N for the limit of quantitation (LOQ) and limit of detection (LOD) are 10:1 and 3:1, respectively (4). The LOD by MS and LOQ by FID for ethanol are 0.005 and 0.010 g%, respectively.

0.005 g%

Matrix	Concentration (g%)	Accuracy	S/N	
			FID	MS
Aqueous	0.0058	16.00%	24.1:1	3.5:1
Urine	0.0061	22.00%	39.3:1	3.8:1
Whole Blood	0.0047	-6.00%	48.9:1	4.2:1

0.010 g%

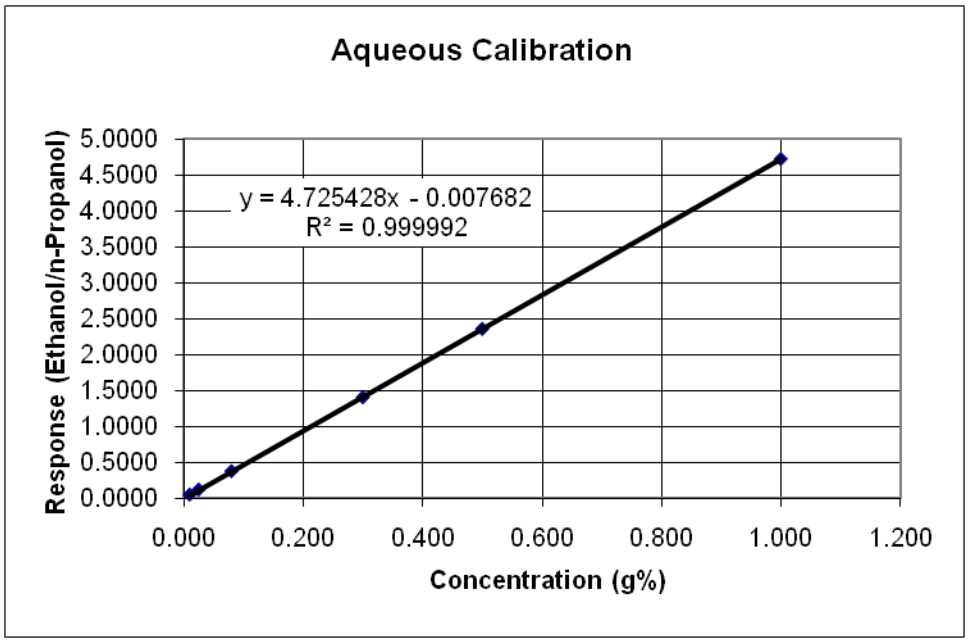
Matrix	Concentration (g%)	Accuracy	S/N	
			FID	MS
Aqueous	0.0106	6.00%	57.7:1	12.3:1
Urine	0.0108	8.00%	46.1:1	10.5:1
Whole Blood	0.0091	-9.00%	55.8:1	10.0:1

Conclusion

The observed sensitivity of the method is more than sufficient for routine casework. Routine calibration will be conducted down to 0.020 g% and an administrative reporting limit of 0.010 g% will be used.

4. Linearity

The aqueous ethanol standards as described above were analyzed. The method was shown to be linear from 0.010 to 1.000 g% with a coefficient of determination (r^2) of 0.999992. The calibration curve that was generated is displayed below.

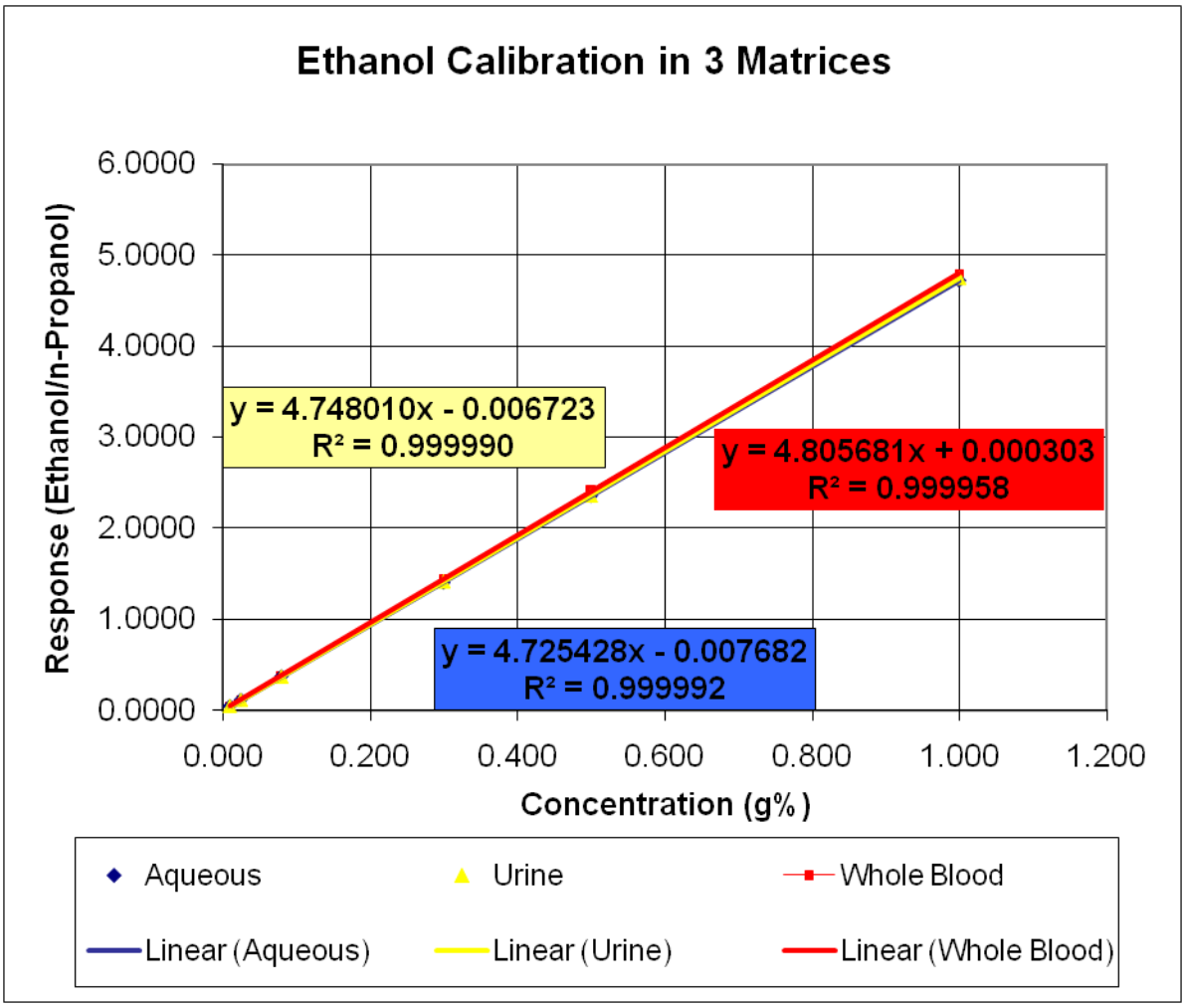


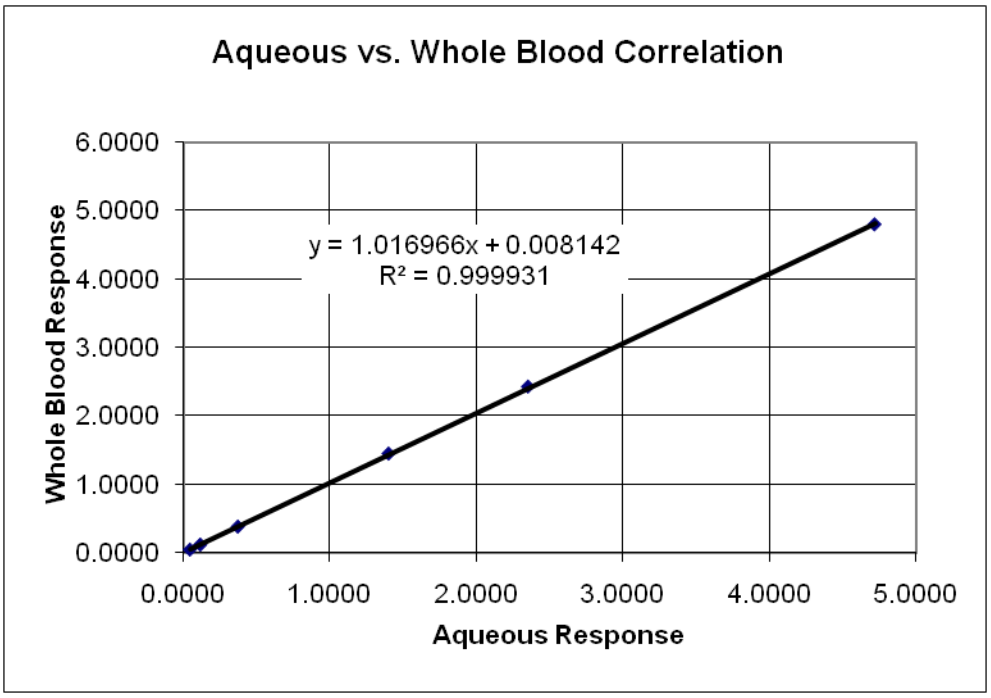
Conclusion

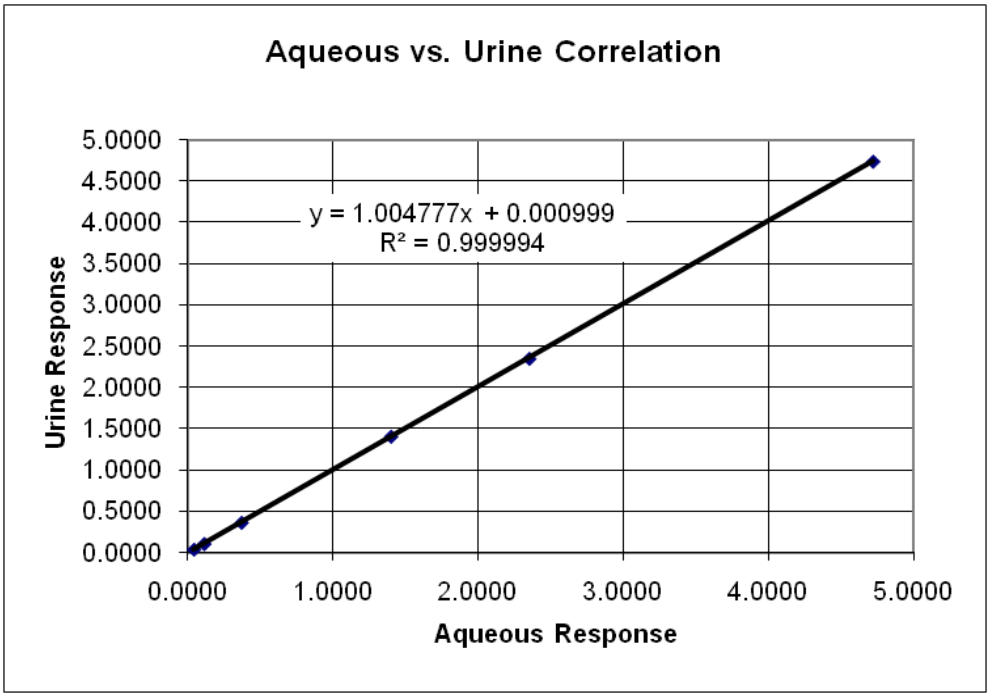
The method is linear from 0.010 to 1.000 g% and is suitable for the typical calibration used for casework (0.020 to 0.500 g%).

5. Evaluation of Matrix Effects

Standards prepared in three matrices (deionized water, urine, and whole blood) over a concentration range of 0.005 to 1.000 g% were analyzed using the procedure to evaluate matrix effects. The calibration curves generated from each matrix were virtually identical when comparing the slope, y-intercept, and r^2. Correlation between the instrument responses for whole blood and urine standards were also compared to aqueous standards by plotting the instrument response (ethanol/n-propanol response ratio) and evaluating the coefficient of determination of the resulting curve. Good correlation was observed for both blood ($r^2 = 0.999931$) and urine ($r^2 = 0.999994$) as compared to aqueous.


Ethanol Calibration in 3 Matrices
Response (Ethanol/n-Propanol)
6.0000
5.0000
4.0000
3.0000
2.0000
1.0000
0.0000
0.000
0.200
0.400
0.600
0.800
1.000
1.200
Concentration (g%)
y = 4.748010x - 0.006723
R² = 0.999990
y = 4.805681x + 0.000303
R² = 0.999958
y = 4.725428x - 0.007682
R² = 0.999992
Aqueous
Urine
Whole Blood
Linear (Aqueous)
Linear (Urine)
Linear (Whole Blood)





Conclusion

There were no observed matrix effects between water and whole blood or water and urine standards. Therefore purchased aqueous ethanol standards may be used as calibrators and controls when analyzing whole blood or urine samples.

6. Evaluation of Carryover

Matrix matched internal standard blank controls were prepared and analyzed immediately after the 1.0 g% prepared standard for each of the 3 matrices. No carryover of ethanol was observed in any of the matrices studied either due to sampling with the dilutor dispenser or analyzing with the instrument.

Conclusion

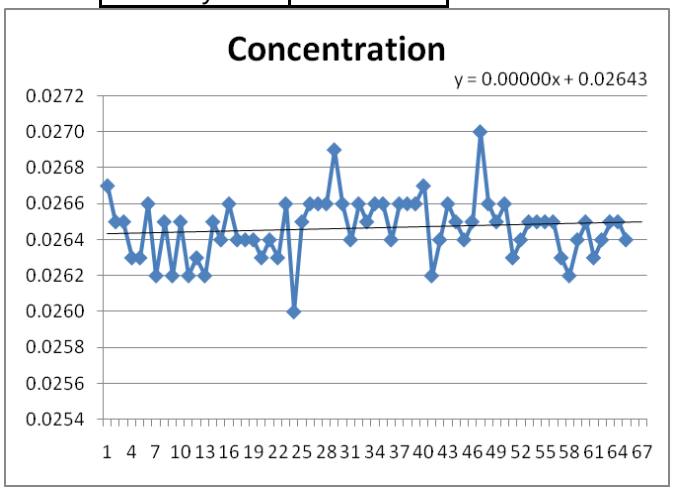
The possibility of carryover was evaluated for both the sampling process as well as during analysis by the instrument. No carryover of ethanol was observed due to either sampling or analyzing matrix matched blank samples immediately after a 1.000 g% ethanol standard for any of the matrices studied (human whole blood, human urine, deionized water). In PBSO casework no sample has ever had a concentration greater than 0.500 g%. Therefore calibrators, controls, and case samples may be run consecutively without blanks or additional rinsing in between.

7. Drift/Bias

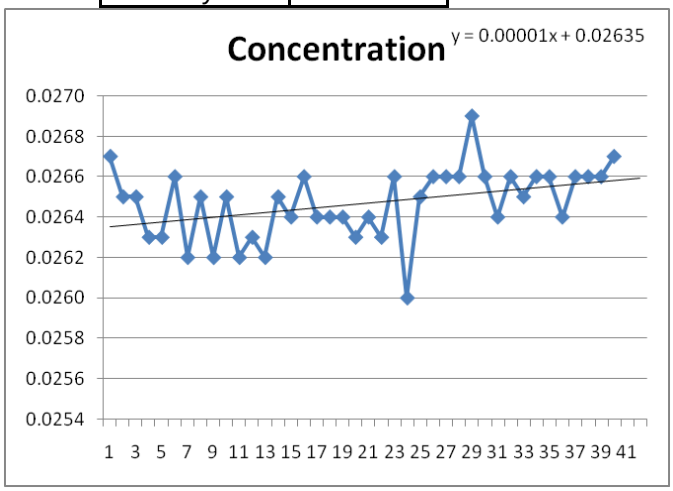
The headspace autosampler used in this method has 70 vial positions. An internal standard blank followed by four calibrators was analyzed along with 65 replicates of each of the controls that will be routinely used for casework (0.025 and 0.300 aqueous from Cerilliant and 0.080 whole blood from Cliniqa). Limiting the number of vials to 45 (40 replicates with 1 blank and 4 calibrators) was compared to the full 70 vials.

Cerilliant 0.025 g%

Level	0.025
Concentration	0.0250
n	65
Mean	0.0265
Minimum	0.0260
Maximum	0.0270
SD	0.0002
CV	0.6352%
Accuracy*	5.8585%

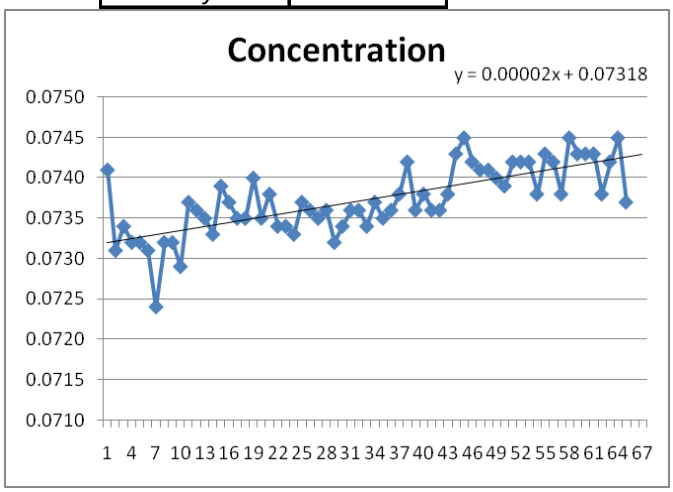


Level	0.025
Concentration	0.0250
n	40
Mean	0.0265
Minimum	0.0260
Maximum	0.0269
SD	0.0002
CV	0.6649%
Accuracy*	5.8700%

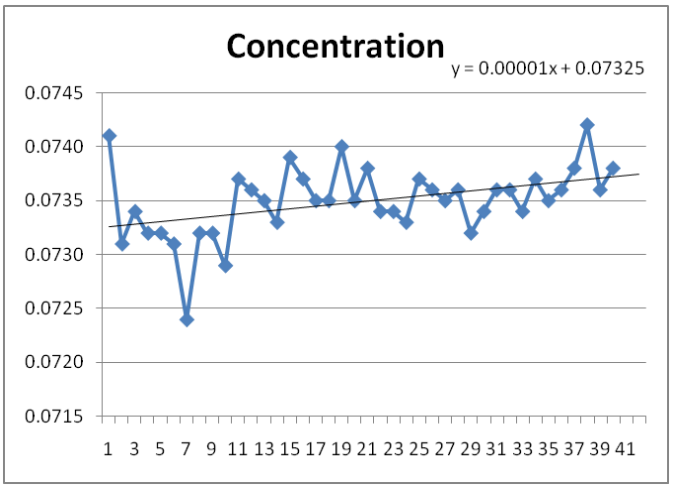


Cliniqa 0.080 g%

Level	0.080
Concentration	0.0755
n	65
Mean	0.0737
Minimum	0.0724
Maximum	0.0745
SD	0.0004
CV	0.5785%
Accuracy*	-2.3515%

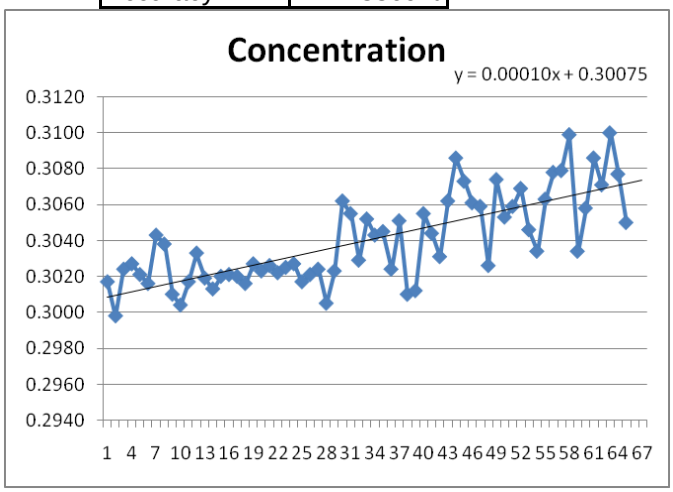


Level	0.080
Concentration	0.0755
n	40
Mean	0.0735
Minimum	0.0724
Maximum	0.0742
SD	0.0003
CV	0.4480%
Accuracy	-2.6589%

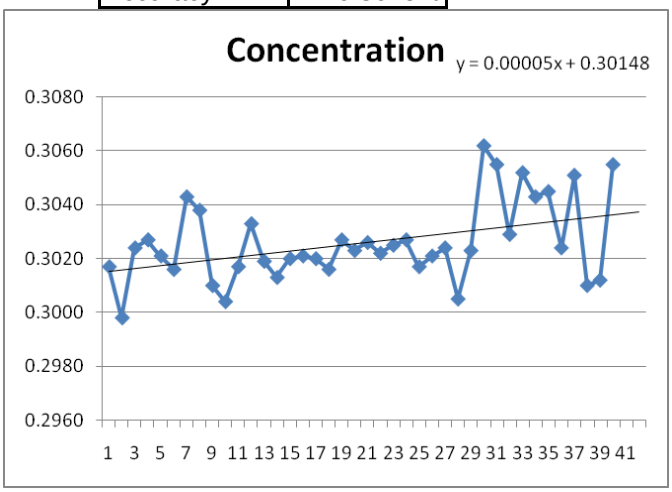


Cerilliant 0.300 g%

Level	0.300
Concentration	0.3000
n	65
Mean	0.3040
Minimum	0.2998
Maximum	0.3100
SD	0.0025
CV	0.8176%
Accuracy*	1.3369%



Level	0.300
Concentration	0.3000
n	40
Mean	0.3026
Minimum	0.2998
Maximum	0.3062
SD	0.0015
CV	0.4934%
Accuracy*	0.8625%



*Accuracy determined by comparing to certified concentration of Cerilliant standards and verified concentration of Cliniqa standard.

Positive drift/bias was observed when running 65 replicates and was significant only at 0.300 g%, causing the minimum and maximum observed concentrations to be outside the precision requirement of ±0.01 g%. By limiting the total batch size to 45 (40 replicates) the drift was minimized and acceptable minimum and maximum ranges were observed at all three levels.

Conclusion

For casework no more than 40 vials will be analyzed in any one batch of samples, with two replicates of each positive level of quality control material analyzed. One replicate at each positive level will be analyzed prior to case samples and one replicate at each positive level will be analyzed after case samples.

8. Reproducibility

Replicates of prepared standards were analyzed to verify the precision and accuracy of the method. The stated criterion in the SOP for precision is that replicate samples must agree within ±0.01 g%. The stated criteria in the SOP for accuracy are that the experimental value must be within ±0.005 g% or ±5% (whichever is larger) of the true value. Recommended precision and accuracy of quality control materials is ±15% and ±20% (or ±30% at or near the concentration of the LOQ), respectively (3). The 0.025, 0.080, and 0.300 g% whole blood prepared standards were analyzed a total of 40 times each (10 replicates of each level by each analyst on four different days) to evaluate between-run and within-run precision and accuracy. Additionally, 65 replicates of each of the controls that will be routinely used for casework (0.025 and 0.300 aqueous from Cerilliant and 0.075 whole blood from Clinqa) were analyzed. The data from the 65 replicates for within-run precision and accuracy has been limited to 40 replicates as described above for the drift/bias evaluation. Complete data for the 65 replicates is presented above in the drift/bias evaluation.

Within-Run Precision / Accuracy of prepared Whole Blood Controls

6/1/10

Level	0.025	0.080	0.300
Concentration*	0.0258	0.0796	0.2980
n	10	10	10
Mean	0.0253	0.0789	0.2972
Minimum	0.0251	0.0786	0.2963
Maximum	0.0254	0.0791	0.2977
SD	0.0001	0.0002	0.0004
CV	0.3727%	0.2349%	0.1430%
Accuracy*	-1.9380%	-0.8920%	-0.2852%

6/2/10

Level	0.025	0.080	0.300
Concentration*	0.0258	0.0796	0.2980
n	10	10	10
Mean	0.0251	0.0786	0.2960
Minimum	0.0250	0.0784	0.2952
Maximum	0.0253	0.0788	0.2967
SD	0.0001	0.0001	0.0005
CV	0.4200%	0.1877%	0.1601%
Accuracy*	-2.7132%	-1.2312%	-0.6611%

6/3/10

Level	0.025	0.080	0.300
Concentration*	0.0258	0.0796	0.2980
n	10	10	10
Mean	0.0253	0.0785	0.2964
Minimum	0.0252	0.0782	0.2945
Maximum	0.0254	0.0788	0.2979
SD	0.0001	0.0002	0.0010
CV	0.3250%	0.2326%	0.3542%
Accuracy*	-1.8217%	-1.3819%	-0.5436%

6/4/10

Level	0.025	0.080	0.300
Concentration*	0.0258	0.0796	0.2980
n	10	10	10
Mean	0.0250	0.0778	0.2938
Minimum	0.0249	0.0776	0.2928
Maximum	0.0251	0.0780	0.2951
SD	0.0000	0.0001	0.0006
CV	0.1886%	0.1539%	0.2169%
Accuracy*	-3.1008%	-2.2487%	-1.4027%

**Concentration determined by analyzing against certified standards on 5/25/10*

Between-Run Precision / Accuracy of prepared Whole Blood Controls

6/1-6/4/10

Level	0.025	0.080	0.300
Concentration*	0.0258	0.0796	0.2980
n	40	40	40
Mean	0.0252	0.0785	0.2958
Minimum	0.0249	0.0776	0.2928
Maximum	0.0254	0.0791	0.2979
SD	0.0002	0.0004	0.0014
CV	0.6415%	0.5507%	0.4794%
Accuracy*	-2.3934%	-1.4384%	-0.7232%

**Concentration determined by analyzing against certified standards on 5/25/10*

Within-Run Precision / Accuracy of purchased controls used for casework limited to 40 replicates

Cerilliant 0.025 g%

Level	0.025
Concentration	0.0250
n	40
Mean	0.0265
Minimum	0.0260
Maximum	0.0269
SD	0.0002
CV	0.6649%
Accuracy	5.8700%

Cliniqa 0.080 g%

Level	0.080
Concentration	0.0755
n	40
Mean	0.0735
Minimum	0.0724
Maximum	0.0742
SD	0.0003
CV	0.4480%
Accuracy	-2.6589%

Cerilliant 0.300 g%

Level	0.300
Concentration	0.3000
n	40
Mean	0.3026
Minimum	0.2998
Maximum	0.3062
SD	0.0015
CV	0.4934%
Accuracy	0.8625%

The coefficient of variation (CV) for within-run and between-run precision for all levels was less than 0.7 %. The precision requirement for the method is that duplicate results should agree within ±0.01 g%. The largest observed difference between the minimum and maximum of the replicates was 0.007 g% at the 0.300 g% level. Quantitative accuracy was within ± 6%, ± 3%, and ± 1.5% for the 0.025, 0.080, and 0.300 g% levels, respectively. All quantitative results were within the stated acceptable ranges (± 0.005 or ± 5%, whichever is larger).

Conclusion

The actual precision and accuracy of the method are well within the stated values across the calibration range (0.020 to 0.500 g%).

9. Specificity

Several volatiles having similar properties to ethanol were prepared and analyzed along with internal standard blanks prepared with three different matrices (deionized water, urine, and whole blood) to verify the specificity of the method for ethanol and the internal standard n-propanol.

Volatile	Retention Time (min)	
	FID	MS
1,1-Difluoroethane	1.638	1.647
Desflurane (breakdown product)	1.936	1.951
Methanol	1.939	1.943
Acetaldehyde	2.160	2.173
Ethanol	2.467	2.478
Sevoflurane	2.733	2.750
Pentane	2.733	2.741
Isopropanol	2.924	2.939
Isoflurane	2.972	2.988
Methylene Chloride	3.169	3.186
Acetone	3.345	3.359
Acetonitrile	3.348	3.359
n-Propanol	3.561	3.573
Hexanes (n-Hexane)	3.836	3.853
Chloroform	4.067	4.091
Ethyl Acetate	4.495	4.511
1-Chlorobutane	4.700	4.709
Heptane	5.055	5.063
Iso Amyl Alcohol	5.894	5.911
Toluene	6.438	6.454
n-Butyl Acetate	8.125	8.142

None of the compounds studied interfered with ethanol or n-propanol. There were no matrix interferences observed in the internal standard blanks prepared in three different matrices.

Conclusion

The combination of headspace sampling, gas chromatography (DB-ALC1 column) and dual detection by FID and MS provides for the specific identification of the target, ethanol, and the internal standard, n-propanol, as well as the other volatiles studied.

10. Reportable Range

The method has a linear range of 0.010 to 1.0 g%. The typical calibration is 0.020 to 0.500 g%.

Conclusion

When results are outside the calibration range (currently 0.020 to 0.500 g%) then quantitative results are not reported. If the result is less than 0.010 g% then the result will be reported as none detected or other similar language, which is consistent with the currently approved method (SOP 785.734.3). If the result is between 0.010 and 0.020 g% then the result will be reported as less than 0.020 g%. If the result is higher than 0.500 g% then the sample will be reported as greater than 0.500 g%. Analysis of standards prepared in three matrices (urine, whole blood, and aqueous) from 0.005 to 1.0 g% demonstrated that the reportable range is within the capabilities of this method.

11. Case comparison (Crossover study)

Eighty-one blood samples were analyzed by this method, 59 of which had quantitative ethyl alcohol results obtained by the currently approved method (SOP 785.734.3). All qualitative results were identical. All quantitative results showed good correlation (quantitative values agreed within ±0.0068 g%). The difference in duplicate measurements of the same sample was within ±0.0042 g% for the HS-GCMS and within ±0.0045 for PE-2. Twenty-two case samples negative for ethyl alcohol did not show any interference with the target analytes.

Case #	PE-2	HS-GCMS	Δ	% Difference
	0.1949	0.1954	0.0005	0.26%
[redacted]	0.1946	0.1931	-0.0015	-0.77%
	0.2592	0.2584	-0.0008	-0.31%
[redacted]	0.2573	0.2583	0.0010	0.39%
	0.1605	0.1586	-0.0019	-1.18%
[redacted]	0.1605	0.1584	-0.0021	-1.31%
	0.1436	0.1413	-0.0023	-1.60%
[redacted]	0.1448	0.1424	-0.0024	-1.66%
	0.0331	0.0293	-0.0038	-11.48%
[redacted]	0.0333	0.0294	-0.0039	-11.71%
	0.3198	0.3151	-0.0047	-1.47%
ATP-1038	0.3200	0.3162	-0.0038	-1.19%
	0.1769	0.1753	-0.0016	-0.90%
ATP-1104	0.1758	0.1756	-0.0002	-0.11%
	0.1775	0.1746	-0.0029	-1.63%
ATP-1084	0.1776	0.1746	-0.0030	-1.69%
	0.3193	0.3152	-0.0041	-1.28%
ATP-1020	0.3187	0.3182	-0.0005	-0.16%
	0.1772	0.1761	-0.0011	-0.62%
ATP-1110	0.1773	0.1759	-0.0014	-0.79%
	0.2360	0.2354	-0.0006	-0.25%
ATP-1177	0.2357	0.2362	0.0005	0.21%
	0.3197	0.3149	-0.0048	-1.50%
ATP-1037	0.3188	0.3176	-0.0012	-0.38%
	0.1772	0.1768	-0.0004	-0.23%
ATP-1103	0.1770	0.1742	-0.0028	-1.58%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.1789	0.1782	-0.0007	-0.39%
[redacted]	0.1784	0.1782	-0.0002	-0.11%
	0.1500	0.1432	-0.0068	-4.53%
[redacted]	0.1488	0.1441	-0.0047	-3.16%

	0.2661	0.2624	-0.0037	-1.39%
[redacted]	0.2685	0.2621	-0.0064	-2.38%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.1561	0.1542	-0.0019	-1.22%
[redacted]	0.1564	0.1562	-0.0002	-0.13%
	0.1976	0.1940	-0.0036	-1.82%
[redacted]	0.1975	0.1979	0.0004	0.20%
	0.2162	0.2140	-0.0022	-1.02%
[redacted]	0.2145	0.2145	0.0000	0.00%
	0.2372	0.2385	0.0013	0.55%
[redacted]	0.2365	0.2388	0.0023	0.97%
	0.2550	0.2598	0.0048	1.88%
[redacted]	0.2567	0.2558	-0.0009	-0.35%
	0.0313	0.0282	-0.0031	-9.90%
[redacted]	0.0311	0.0288	-0.0023	-7.40%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	

	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.2789	0.2798	0.0009	0.32%
[redacted]	0.2789	0.2784	-0.0005	-0.18%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.2228	0.2254	0.0026	1.17%
[redacted]	0.2237	0.2261	0.0024	1.07%
	0.2386	0.2426	0.0040	1.68%
[redacted]	0.2395	0.2454	0.0059	2.46%
	0.2164	0.2216	0.0052	2.40%
[redacted]	0.2165	0.2174	0.0009	0.42%
	0.0869	0.0885	0.0016	1.84%
[redacted]	0.0861	0.0888	0.0027	3.14%
	0.2747	0.2717	-0.0030	-1.09%
[redacted]	0.2792	0.2726	-0.0066	-2.36%
	0.1859	0.1842	-0.0017	-0.91%
[redacted]	0.1862	0.1841	-0.0021	-1.13%
	0.1485	0.1429	-0.0056	-3.77%
[redacted]	0.1484	0.1423	-0.0061	-4.11%
	0.1761	0.1763	0.0002	0.11%
[redacted]	0.1772	0.1758	-0.0014	-0.79%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.1682	0.1676	-0.0006	-0.36%
[redacted]	0.1692	0.1680	-0.0012	-0.71%
	0.0719	0.0714	-0.0005	-0.70%
[redacted]	0.0717	0.0712	-0.0005	-0.70%
	0.2301	0.2287	-0.0014	-0.61%
[redacted]	0.2301	0.2274	-0.0027	-1.17%
	0.3463	0.3456	-0.0007	-0.20%
[redacted]	0.3491	0.3451	-0.0040	-1.15%
	0.1626	0.1618	-0.0008	-0.49%
[redacted]	0.1611	0.1591	-0.0020	-1.24%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	

	0.1659	0.1678	0.0019	1.15%
[redacted]	0.1666	0.1677	0.0011	0.66%
	0.3480	0.3467	-0.0013	-0.37%
[redacted]	0.3484	0.3469	-0.0015	-0.43%
	0.2989	0.3007	0.0018	0.60%
[redacted]	0.2987	0.2983	-0.0004	-0.13%
	0.2377	0.2367	-0.0010	-0.42%
[redacted]	0.2376	0.2370	-0.0006	-0.25%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0978	0.0980	0.0002	0.20%
[redacted]	0.0975	0.0986	0.0011	1.13%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.1411	0.1409	-0.0002	-0.14%
[redacted]	0.1406	0.1409	0.0003	0.21%
	0.0990	0.0996	0.0006	0.61%
[redacted]	0.0990	0.0997	0.0007	0.71%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0814	0.0814	0.0000	0.00%
[redacted]	0.0815	0.0814	-0.0001	-0.12%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.1195	0.1203	0.0008	0.67%
[redacted]	0.1197	0.1198	0.0001	0.08%
	0.1288	0.1296	0.0008	0.62%
[redacted]	0.1289	0.1299	0.0010	0.78%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0180	0.0159	-0.0021	-11.67%
[redacted]	0.0179	0.0161	-0.0018	-10.06%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	

	0.2201	0.2208	0.0007	0.32%
[redacted]	0.2206	0.2244	0.0038	1.72%
	0.2247	0.2242	-0.0005	-0.22%
[redacted]	0.2243	0.2242	-0.0001	-0.04%
	0.1734	0.1745	0.0011	0.63%
[redacted]	0.1735	0.1753	0.0018	1.04%
	0.0000	0.0000	0.0000	
[redacted]	0.0000	0.0000	0.0000	
	0.0889	0.0888	-0.0001	-0.11%
[redacted]	0.0885	0.0887	0.0002	0.23%
	0.0757	0.0758	0.0001	0.13%
ATP-1081	0.0759	0.0756	-0.0003	-0.40%
	0.1805	0.1820	0.0015	0.83%
ATP-1006	0.1812	0.1830	0.0018	0.99%
	0.1810	0.1830	0.0020	1.10%
ATP-1027	0.1819	0.1821	0.0002	0.11%
	0.0753	0.0758	0.0005	0.66%
ATP-1111	0.0747	0.0756	0.0009	1.20%
	0.1818	0.1827	0.0009	0.50%
ATP-1010	0.1823	0.1845	0.0022	1.21%
	0.0756	0.0758	0.0002	0.26%
ATP-1090	0.0757	0.0756	-0.0001	-0.13%
	0.0761	0.0756	-0.0005	-0.66%
ATP-1087	0.0762	0.0757	-0.0005	-0.66%
	0.2562	0.2571	0.0009	0.35%
ATP-1187	0.2574	0.2568	-0.0006	-0.23%

Conclusion

The method showed good correlation with the currently approved method for quantitative ethyl alcohol determination in proficiency and case samples.

12. Competency Testing

Nick Tiscione, Xiaoqin Shan, Ilene Alford and Tate Yeatman each performed at least one of the experiments used to validate this method, and by doing so demonstrated their competency in performance of the method. Each analyst performed all steps of the experiments they were involved with including sample preparation, analysis, and data processing.

Conclusion

All analysts currently in the Toxicology Unit have demonstrated their competency in using this method.

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