Method Validation For Quantitative Test Methods

Analyte	***Ethanol***
Units of Measure	***g/100 mL***
Analyst Performing Validation Studies	***Matthew A. Cheney, Ph.D.***
Analyst Performing Data Analysis	***Corissa L. Rodgers, M.S.***
Responsible Supervisor	***Dayong Lee, Ph.D.***
Start Date	***May 4, 2016***
Completion Date	***May 12, 2016***
Primary Matrix	***Whole Blood***
Secondary Matrices	***Serum, Plasma, Alcoholic Beverages, Other Liquid Specimens***
Low Calibrator Concentration	***0.010 g/100 mL***
Highest Calibrator Concentration	***0.500 g/100 mL***
Equipment/Instrument	***Headspace 3 - FID1***
Instrument Serial Numbers	***Headspace CN16140002, Gas Chromatograph US16163003***
Method	***VOLATILES.M***
Validation Report Date	***May 17, 2016***

Validation Approval

Analyst: Matthew Andrade Cheney, Ph.D. Digitally signed by Matthew Andrade Cheney, Ph.D. DN: cn=Matthew Andrade Cheney, Ph.D., o=Houston Forensic Science Center, ou=FAD - Toxicology, email=macheney@houstonforensicscience.org, c=US Date: 2016.05.17 12:12:49 -05'00' 2016-05-17
Date

Analyst: Corissa L. Rodgers, M.S. Digitally signed by Corissa L. Rodgers, M.S. DN: cn=Corissa L. Rodgers, M.S., o=Houston Forensic Science Center, ou=FAD - Toxicology, email=crodgers@houstonforensicscience.org, c=US Date: 2016.05.17 11:57:23 -05'00' 2016-05-17
Date

Responsible Supervisor: Dayong Lee Digitally signed by Dayong Lee DN: cn=Dayong Lee, o=Houston Forensic Science Center, ou=Toxicology, email=dlee@houstonforensicscience.org, c=US Date: 2016.05.17 13:02:17 -05'00' 2016-05-17
Date

Quality Director: Lori Wilson Digitally signed by Lori Wilson DN: cn=Lori Wilson, o, ou, email=lwilson@houstonforensicscience.org, c=US Date: 2016.05.17 15:05:42 -05'00' 5/17/2016
Date

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: ***Ethanol***
Units: ***g/100 mL***
Method: ***VOLATILES.M***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

	STUDY	SOP CRITERIA	ALTERNATE CRITERIA*	RESULTS	VALIDATION COMMENTS
1	Standard Curve / Calibration	Weighting minimizes Σ|%RE| 95% CI of slope includes 1 95% CI of intercept includes 0 95% CI of slope includes 1 95% CI of intercept includes 0	R^2 value ≥0.99 %RE Calibrators: ≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL	Unweighted: = 65.29 1/x Weighting: = 39.54 1/x2 Weighting: = 38.33 95% CI of slope = 1.0003 - 1.0039 95% CI of Intercept = -0.0008 - 0.0001 Max %RE low calibrator = 2.00 Max %RE other calibrators = 0.72 N/A	1/x weighting chosen. Upon inspection of the standardized residual plot, the Day 3, 0.500 g/100 mL calibrator was determined to be a statistically significant outlier. If this data point is excluded, 95% CI of the slope contains 1. Linearity is deemed acceptable. Not performed; a 7-point calibration curve will be used for ethanol casework analysis.
2	Limit of Detection (LOD)	Signal to Noise ≥ 3 Acceptable detection and identification criteria		LOD = 0.0025 S:N = 251.902	LOD samples were made using three sources of blank blood, W044616241086 (A), W0446162247837 (B), and W044616247845 (C). Theoretical target concentrations for LOD samples were 0.0025, 0.0050, and 0.0075 g/100 mL, using 0.125, 0.250, and 0.375 mL of 1000 µg/mL mixed volatile standard (lot FN09231404), respectively, diluted with each blood source to 5 mL.
3	Limit of Quantification (LOQ)		Bias: ≤ ± 10% Within-Run Imprecision: CV ≤ ± 10% Between-Run Imprecision: CV ≤ ± 10%	Bias = 0.53% Within-run Imprecision= 1.01% Between-Run Imprecision = 0.38%	Theoretical target concentration for LOQ (lot 20160503-LOQ) is 0.0100 g/100 mL, using 1.0 mL of 1000 µg/mL mixed volatile standard (lot FN09231404) diluted with drug-free pooled blood to 10 mL.
4	Bias Within-Run Imprecision Between-Run Imprecision		Bias: ≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL Within-Run Imprecision: CV ≤ ± 10% Between-Run Imprecision: CV ≤ ± 10%	Max Bias = 4.72% Max Within-Run Imprecision = 0.57% Max Within-Run Imprecision = 0.51%	Theoretical target concentration for LMQC (lot 20160503-LMQC) is 0.0192 g/100 mL, using 1.2 mL 4000 µg/mL mixed volatile standard (lot FN01301503) diluted with deionized water to 25 mL. Target concentrations for MQC1 (lot 1304030) and MQC2 (lot 1407168) were established during this validation. Consequently, the bias for MQC1 and MQC2 could have been underestimated. However, as LMQC uses a nominal concentration as the target and still shows acceptable bias, performance of this method is deemed fit for purpose.
5	Carryover	Quantitative result ≤ 25% of LOQ		Maximum Carryover = 0.00%	Carryover evaluated by following the highest calibrator with a negative.
6	Matrix Interference Internal Standard Interference Exogenous Substances Interferences	Response of blank matrix ≤ 10% of the average response of LOQ Responses 10% of the average response of LOQ Concentration of analytes of interest meet acceptance criteria of the method.		None observed. None observed. No compounds tested caused significant interference with the analyte of interest.	Toluene and ethyl acetate: mixed 500 µL of each reagent with 500 µL of deionized water, LMQC (lot 20160503-LMQC) and internal standard (20160429-I) were added to a headspace vial, 5 µL of either toluene or ethyl acetate diluted solution immediately added, and vials capped. Sharpie marker: the bottom of a headspace vial was mostly marked with Sharpie marker, LMQC and internal standard added immediately afterward, and vial capped. VWR marker: a small piece of Kim Wipe was extensively marked with VWR marker, added to a headspace vial, LMQC and internal standard added immediately afterward, and vial capped. *The Sharpie® Permanent Marker contained n-propanol, which more than quadrupled the internal standard signal and thus diminished the calculated value of the analyte of interest.
7	Dilution of Samples		≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL	2x Max % of Target = -0.73% 20x Max % of Target = -6.97% 50x Max % of Target = -9.63% 100x Max % of Target = -10.39%	The target concentration for MQC2 (lot 1407168) is based on the mean value obtained from the bias and precision study. The target concentration for the remaining dilutions is the concentration of the ethanol standard (lot 097K5012V; converted 10% v/v to w/v using the density of ethanol to obtain 7.890 g/100 mL). Analytical results were multiplied by the dilution factors to give the calculated value and compared to the target concentration. Case samples can be diluted by 1:2 prior to analysis. The higher dilution factors of 1:20, 1:50, and 1:100 will underestimate the ethanol concentration by up to approximately 7%, 10%, and 11%, respectively. If used, this method limitation will be disclosed on the test report.
8	In-Process Stability Autosampler Stability Storage Stability		≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL ≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL ≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL	N/A Samples on the autosampler may be analyzed for ethanol up to 72 hours after preparation. N/A	Study not performed; it is not appropriate to break for extended periods during sample preparation. Study not performed; the stability of ethanol in storage conditions is well documented in the literature.
9	Matrix Matching		≤ ± 5% if target concentration is >0.05 g/100 mL ≤ ± 10% if target concentration is ≤0.05 g/100 mL % CV ≤ ± 10%		Study not performed; all samples were diluted 10x before analysis.

*** Alternate criteria is any deviation from that described in the SOP as determined by the Supervisor**

ADDITIONAL STUDIES (Describe any additional studies required for this validation.)

A qualitative study was performed to determine the retention time of acetaldehyde, a metabolite of ethanol, using this method. The retention time of acetaldehyde is 0.940 minutes on FID1.

Validation Study 1 — **STANDARD CURVE WEIGHT VERIFICATION**

Analyte: ***Ethanol*** | Analyst: ***Matthew A. Cheney, Ph.D.***

Units: ***g/100 mL*** | Study Dates: ***05/04/16 to 05/12/16***

Instrument: ***Headspace 3 - FID1*** | Primary Matrix: ***Whole Blood***

	Σ|%RE|
Unweighted:	65.29
1/x Weighting:	39.54
1/x^2 Weighting:	38.33

Comments: 1/x weighting chosen.

Acceptance Criteria: **Weighting minimizes Σ|%RE|**

Cnom	x	y	w	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.1	0.051283240	1	0.005128	0.1	0.05128324	0.01	0.00263	0.010464	4.64072	4.64072
0.025	0.25	0.127368840	1	0.031842	0.25	0.12736884	0.0625	0.016223	0.024874	-0.50263	0.502631
0.05	0.5	0.257239844	1	0.12862	0.5	0.25723984	0.25	0.066172	0.049471	-1.05735	1.057348
0.1	1	0.513586023	1	0.513586	1	0.51358602	1	0.263771	0.098022	-1.97786	1.977861
0.2	2	1.034654430	1	2.069309	2	1.03465443	4	1.07051	0.19671	-1.64492	1.644925
0.4	4	2.068710443	1	8.274842	4	2.06871044	16	4.279563	0.392556	-1.86107	1.861073
0.5	5	2.607575305	1	13.03788	5	2.60757531	25	6.799449	0.494614	-1.07714	1.077142
0.01	0.1	0.051389058	1	0.005139	0.1	0.05138906	0.01	0.002641	0.010484	4.841135	4.841135
0.025	0.25	0.128311248	1	0.032078	0.25	0.12831125	0.0625	0.016464	0.025053	0.211321	0.211321
0.05	0.5	0.258426478	1	0.129213	0.5	0.25842648	0.25	0.066784	0.049696	-0.60786	0.607861
0.1	1	0.514412955	1	0.514413	1	0.51441296	1	0.264621	0.098179	-1.82124	1.821244
0.2	2	1.035780376	1	2.071561	2	1.03578038	4	1.072841	0.196923	-1.5383	1.5383
0.4	4	2.081327018	1	8.325308	4	2.08132702	16	4.331922	0.394945	-1.26369	1.263693
0.5	5	2.609883357	1	13.04942	5	2.60988336	25	6.811491	0.495051	-0.98972	0.989716
0.01	0.1	0.052562809	1	0.005256	0.1	0.05256281	0.01	0.002763	0.010706	7.064167	7.064167
0.025	0.25	0.130661973	1	0.032665	0.25	0.13066197	0.0625	0.017073	0.025498	1.992188	1.992188
0.05	0.5	0.262247687	1	0.131124	0.5	0.26224769	0.25	0.068774	0.05042	0.839578	0.839578
0.1	1	0.522798035	1	0.522798	1	0.52279804	1	0.273318	0.099767	-0.23315	0.233147
0.2	2	1.048294622	1	2.096589	2	1.04829462	4	1.098922	0.199294	-0.35323	0.353229
0.4	4	2.107942670	1	8.431771	4	2.10794267	16	4.443422	0.399986	-0.00347	0.003471
0.5	5	2.671933428	1	13.35967	5	2.67193343	25	7.139228	0.506803	1.360685	1.360685
0.01	0.1	0.052673507	1	0.005267	0.1	0.05267351	0.01	0.002774	0.010727	7.273823	7.273823
0.025	0.25	0.131320158	1	0.03283	0.25	0.13132016	0.0625	0.017245	0.025623	2.490817	2.490817
0.05	0.5	0.264264500	1	0.132132	0.5	0.2642645	0.25	0.069836	0.050802	1.603529	1.603529
0.1	1	0.529964740	1	0.529965	1	0.52996474	1	0.280863	0.101124	1.124195	1.124195
0.2	2	1.060104810	1	2.12021	2	1.06010481	4	1.123822	0.20153	0.765169	0.765169
0.4	4	2.115537260	1	8.462149	4	2.11553726	16	4.475498	0.401424	0.356124	0.356124
0.5	5	2.665470599	1	13.32735	5	2.6654706	25	7.104734	0.505579	1.115879	1.115879
0.01	0.1	0.052755682	1	0.005276	0.1	0.05275568	0.01	0.002783	0.010743	7.42946	7.42946
0.025	0.25	0.131023657	1	0.032756	0.25	0.13102366	0.0625	0.017167	0.025567	2.266193	2.266193
0.05	0.5	0.264342378	1	0.132171	0.5	0.26434238	0.25	0.069877	0.050817	1.633029	1.633029
0.1	1	0.526926119	1	0.526926	1	0.52692612	1	0.277651	0.100549	0.548693	0.548693
0.2	2	1.057919799	1	2.11584	2	1.0579198	4	1.119194	0.201117	0.558254	0.558254
0.4	4	2.136520374	1	8.546081	4	2.13652037	16	4.564719	0.405399	1.349651	1.349651
0.5	5	2.659675733	1	13.29838	5	2.65967573	25	7.073875	0.504482	0.896375	0.896375
Sum			35	122.0355	64.25	33.7848892	231.6125	64.30862	6.425	35.42934	**65.29**
Slope	0.52799565										
Intercept	-0.00396661										
r^2	0.99986752										

Cnom	x	y	w (1/x)	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.1	0.051283	1	0.005128	0.1	0.05128324	0.01	0.00263	0.00999	-0.09887	0.09887
0.025	0.25	0.127369	0.4	0.012737	0.1	0.05094754	0.025	0.006489	0.02444	-2.24065	2.240648
0.05	0.5	0.25724	0.2	0.025724	0.1	0.05144797	0.05	0.013234	0.049104	-1.79167	1.791666
0.1	1	0.513586	0.1	0.051359	0.1	0.0513586	0.1	0.026377	0.097788	-2.21209	2.21209
0.2	2	1.034654	0.05	0.103465	0.1	0.05173272	0.2	0.053525	0.196746	-1.62694	1.626938
0.4	4	2.06871	0.025	0.206871	0.1	0.05171776	0.4	0.106989	0.393128	-1.71803	1.718026
0.5	5	2.607575	0.02	0.260758	0.1	0.05215151	0.5	0.135989	0.495466	-0.90682	0.906819
0.01	0.1	0.051389	1	0.005139	0.1	0.05138906	0.01	0.002641	0.01001	0.102093	0.102093
0.025	0.25	0.128311	0.4	0.012831	0.1	0.0513245	0.025	0.006586	0.024619	-1.52474	1.524742
0.05	0.5	0.258426	0.2	0.025843	0.1	0.0516853	0.05	0.013357	0.04933	-1.34095	1.340949
0.1	1	0.514413	0.1	0.051441	0.1	0.0514413	0.1	0.026462	0.097945	-2.05504	2.055044
0.2	2	1.03578	0.05	0.103578	0.1	0.05178902	0.2	0.053642	0.19696	-1.52002	1.520021
0.4	4	2.081327	0.025	0.208133	0.1	0.05203318	0.4	0.108298	0.395524	-1.11901	1.11901
0.5	5	2.609883	0.02	0.260988	0.1	0.05219767	0.5	0.13623	0.495904	-0.81915	0.819152
0.01	0.1	0.052563	1	0.005256	0.1	0.05256281	0.01	0.002763	0.010233	2.331212	2.331212
0.025	0.25	0.130662	0.4	0.013066	0.1	0.05226479	0.025	0.006829	0.025065	0.261001	0.261001
0.05	0.5	0.262248	0.2	0.026225	0.1	0.05244954	0.05	0.013755	0.050055	0.110454	0.110454
0.1	1	0.522798	0.1	0.05228	0.1	0.0522798	0.1	0.027332	0.099537	-0.4626	0.4626
0.2	2	1.048295	0.05	0.104829	0.1	0.05241473	0.2	0.054946	0.199337	-0.33171	0.331706
0.4	4	2.107943	0.025	0.210794	0.1	0.05269857	0.4	0.111086	0.400579	0.144662	0.144662
0.5	5	2.671933	0.02	0.267193	0.1	0.05343867	0.5	0.142785	0.507688	1.537684	1.537684
0.01	0.1	0.052674	1	0.005267	0.1	0.05267351	0.01	0.002774	0.010254	2.541442	2.541442
0.025	0.25	0.13132	0.4	0.013132	0.1	0.05252806	0.025	0.006898	0.02519	0.760995	0.760995
0.05	0.5	0.264264	0.2	0.026426	0.1	0.0528529	0.05	0.013967	0.050438	0.876496	0.876496
0.1	1	0.529965	0.1	0.052996	0.1	0.05299647	0.1	0.028086	0.100898	0.898458	0.898458
0.2	2	1.060105	0.05	0.10601	0.1	0.05300524	0.2	0.056191	0.20158	0.789755	0.789755
0.4	4	2.115537	0.025	0.211554	0.1	0.05288843	0.4	0.111887	0.402021	0.505242	0.505242
0.5	5	2.665471	0.02	0.266547	0.1	0.05330941	0.5	0.142095	0.506461	1.292207	1.292207
0.01	0.1	0.052756	1	0.005276	0.1	0.05275568	0.01	0.002783	0.01027	2.697505	2.697505
0.025	0.25	0.131024	0.4	0.013102	0.1	0.05240946	0.025	0.006867	0.025134	0.535756	0.535756
0.05	0.5	0.264342	0.2	0.026434	0.1	0.05286848	0.05	0.013975	0.050453	0.906076	0.906076
0.1	1	0.526926	0.1	0.052693	0.1	0.05269261	0.1	0.027765	0.100321	0.321381	0.321381
0.2	2	1.05792	0.05	0.105792	0.1	0.05289599	0.2	0.05596	0.201165	0.582272	0.582272
0.4	4	2.13652	0.025	0.213652	0.1	0.05341301	0.4	0.114118	0.406006	1.501489	1.501489
0.5	5	2.659676	0.02	0.265968	0.1	0.05319351	0.5	0.141478	0.505361	1.072102	1.072102
Sum	64.25	33.78489	8.975	3.378489	3.5	1.83109102	6.425	1.776789			**39.53656**
Slope	0.52655397										
Intercept	-0.00132010										
r^2	0.99991116										

Cnom	x	y	w (1/x^2)	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.1	0.051283	10	0.05128324	1	0.512832	0.1	0.0263	0.009871	-1.29122	1.291221
0.025	0.25	0.127369	1.6	0.05094754	0.4	0.20379	0.1	0.025957	0.02438	-2.48	2.480004
0.05	0.5	0.25724	0.4	0.05144797	0.2	0.102896	0.1	0.026469	0.049146	-1.70858	1.708576
0.1	1	0.513586	0.1	0.0513586	0.1	0.051359	0.1	0.026377	0.09803	-1.97043	1.970428
0.2	2	1.034654	0.025	0.05173272	0.05	0.025866	0.1	0.026763	0.197395	-1.30272	1.302721
0.4	4	2.06871	0.00625	0.05171776	0.025	0.012929	0.1	0.026747	0.394584	-1.35411	1.354108
0.5	5	2.607575	0.004	0.05215151	0.02	0.01043	0.1	0.027198	0.497342	-0.53155	0.531551
0.01	0.1	0.051389	10	0.05138906	1	0.513891	0.1	0.026408	0.009891	-1.08943	1.089432
0.025	0.25	0.128311	1.6	0.0513245	0.4	0.205298	0.1	0.026342	0.02456	-1.76115	1.761154
0.05	0.5	0.258426	0.4	0.0516853	0.2	0.103371	0.1	0.026714	0.049372	-1.25601	1.256006
0.1	1	0.514413	0.1	0.0514413	0.1	0.051441	0.1	0.026462	0.098187	-1.81274	1.812737
0.2	2	1.03578	0.025	0.05178902	0.05	0.025895	0.1	0.026821	0.197609	-1.19536	1.195365
0.4	4	2.081327	0.00625	0.05203318	0.025	0.013008	0.1	0.027075	0.396989	-0.75263	0.75263
0.5	5	2.609883	0.004	0.05219767	0.02	0.01044	0.1	0.027246	0.497782	-0.44352	0.443525
0.01	0.1	0.052563	10	0.05256281	1	0.525628	0.1	0.027628	0.010115	1.14885	1.14885
0.025	0.25	0.130662	1.6	0.05226479	0.4	0.209059	0.1	0.027316	0.025008	0.031928	0.031928
0.05	0.5	0.262248	0.4	0.05244954	0.2	0.104899	0.1	0.02751	0.050101	0.201363	0.201363
0.1	1	0.522798	0.1	0.0522798	0.1	0.05228	0.1	0.027332	0.099786	-0.21375	0.213746
0.2	2	1.048295	0.025	0.05241473	0.05	0.026207	0.1	0.027473	0.199996	-0.00216	0.002164
0.4	4	2.107943	0.00625	0.05269857	0.025	0.013175	0.1	0.027771	0.402065	0.516236	0.516236
0.5	5	2.671933	0.004	0.05343867	0.02	0.010688	0.1	0.028557	0.509615	1.922999	1.922999
0.01	0.1	0.052674	10	0.05267351	1	0.526735	0.1	0.027745	0.010136	1.359945	1.359945
0.025	0.25	0.13132	1.6	0.05252806	0.4	0.210112	0.1	0.027592	0.025133	0.533978	0.533978
0.05	0.5	0.264264	0.4	0.0528529	0.2	0.105706	0.1	0.027934	0.050485	0.970554	0.970554
0.1	1	0.529965	0.1	0.05299647	0.1	0.052996	0.1	0.028086	0.101153	1.152906	1.152906
0.2	2	1.060105	0.025	0.05300524	0.05	0.026503	0.1	0.028096	0.202248	1.123906	1.123906
0.4	4	2.115537	0.00625	0.05288843	0.025	0.013222	0.1	0.027972	0.403513	0.878298	0.878298
0.5	5	2.665471	0.004	0.05330941	0.02	0.010662	0.1	0.028419	0.508383	1.676514	1.676514
0.01	0.1	0.052756	10	0.05275568	1	0.527557	0.1	0.027832	0.010152	1.516649	1.516649
0.025	0.25	0.131024	1.6	0.05240946	0.4	0.209638	0.1	0.027468	0.025077	0.307814	0.307814
0.05	0.5	0.264342	0.4	0.05286848	0.2	0.105737	0.1	0.027951	0.0505	1.000256	1.000256
0.1	1	0.526926	0.1	0.05269261	0.1	0.052693	0.1	0.027765	0.100573	0.573457	0.573457
0.2	2	1.05792	0.025	0.05289599	0.05	0.026448	0.1	0.02798	0.201831	0.915571	0.915571
0.4	4	2.13652	0.00625	0.05341301	0.025	0.013353	0.1	0.028529	0.407515	1.87864	1.87864
0.5	5	2.659676	0.004	0.05319351	0.02	0.010639	0.1	0.028296	0.507278	1.455504	1.455504
Sum	64.25	33.78489	60.67625	1.83109102	8.975	4.677382	3.5	0.958129			**38.33**
Slope	0.52439840										
Intercept	-0.00047949										
r^2	0.99987434										

Validation Study 1

LINEARITY

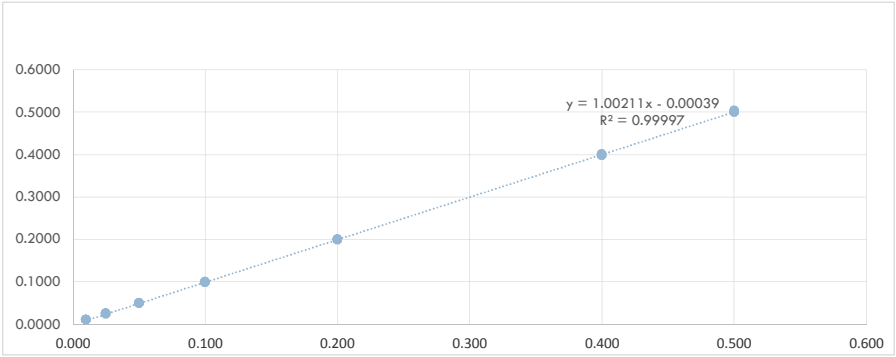
Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

x	y
0.010	0.0102
0.025	0.0248
0.050	0.0498
0.100	0.0994
0.200	0.1999
0.400	0.3992
0.500	0.5018
0.010	0.0102
0.025	0.0249
0.050	0.0500
0.100	0.0989
0.200	0.1994
0.400	0.4003
0.500	0.5013
0.010	0.0102
0.025	0.0250
0.050	0.0498
0.100	0.0987
0.200	0.1987
0.400	0.3990
0.500	0.5036
0.010	0.0101
0.025	0.0249
0.050	0.0499
0.100	0.1000
0.200	0.2001
0.400	0.3991
0.500	0.5009
0.010	0.0101
0.025	0.0248
0.050	0.0499
0.100	0.0994
0.200	0.1993
0.400	0.4023
0.500	0.4991

Slope	1.00211
Standard error in slope, S_b	0.00088
Degrees Freedom	34
Confidence Level	95%
Student t Value	2.03224
Confidence Interval	0.00178
Slope ± CI	1.00211 ± 0.00178
Range	1.0003 - 1.0039
	FAIL

Intercept	-0.00039
Standard error in intercept	0.00023
Degrees Freedom	34
Confidence Level	95%
Student t Value	2.03224
Confidence Interval	0.00046
Intercept ± CI	-0.00039 ± 0.00046
Range	-0.0008 - 0.0001
	PASS



Comments: Upon inspection of the standardized residual plot, the Day 3, 0.500 g/100 mL calibrator was determined to be a statistically significant outlier. If this data point is excluded, 95% CI of the slope contains 1. Linearity is deemed acceptable.

Acceptance Criteria:

95% CI of slope includes 1
95% CI of intercept includes 0

Validation Study 1 — **STANDARD CURVE CALIBRATION**

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

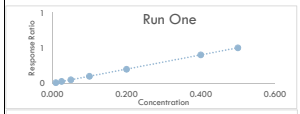
Run Date	Run Order	Target	Result	% Difference from Target
Run 1 05/04/16	Level 1	0.010	0.0102	2.00
	Level 2	0.025	0.0248	-0.80
	Level 3	0.050	0.0498	-0.40
	Level 4	0.100	0.0994	-0.60
	Level 5	0.200	0.1999	-0.05
	Level 6	0.400	0.3992	-0.20
	Level 7	0.500	0.5018	0.36
Run 2 05/05/16	Level 1	0.010	0.0102	2.00
	Level 2	0.025	0.0249	-0.40
	Level 3	0.050	0.0500	0.00
	Level 4	0.100	0.0989	-1.10
	Level 5	0.200	0.1994	-0.30
	Level 6	0.400	0.4003	0.07
	Level 7	0.500	0.5013	0.26
Run 3 05/06/16	Level 1	0.010	0.0102	2.00
	Level 2	0.025	0.0250	0.00
	Level 3	0.050	0.0498	-0.40
	Level 4	0.100	0.0987	-1.30
	Level 5	0.200	0.1987	-0.65
	Level 6	0.400	0.3990	-0.25
	Level 7	0.500	0.5036	0.72
Run 4 05/09/16	Level 1	0.010	0.0101	1.00
	Level 2	0.025	0.0249	-0.40
	Level 3	0.050	0.0499	-0.20
	Level 4	0.100	0.1000	0.00
	Level 5	0.200	0.2001	0.05
	Level 6	0.400	0.3991	-0.23
	Level 7	0.500	0.5009	0.18
Run 5 05/11/16	Level 1	0.010	0.0101	1.00
	Level 2	0.025	0.0248	-0.80
	Level 3	0.050	0.0499	-0.20
	Level 4	0.100	0.0994	-0.60
	Level 5	0.200	0.1993	-0.35
	Level 6	0.400	0.4023	0.57
	Level 7	0.500	0.4991	-0.18

Max %RE LOQ = 2.00
Max %RE = 0.72

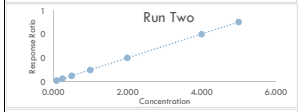
Comments:

Acceptance Criteria:

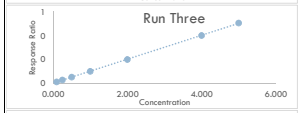
R^2 value ≥0.99
%RE Calibrators:
≤ ± 5% if target concentration is >0.05 g/100 mL
≤ ± 10% if target concentration is ≤0.05 g/100 mL



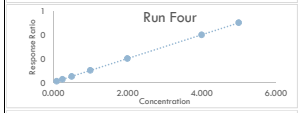
	Weighted
Intercept	1.80752E-05
Slope	0.099997936
r^2	0.999991069



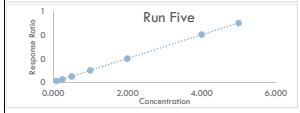
Intercept	3.78446E-05
Slope	0.099979384
r^2	0.999989273



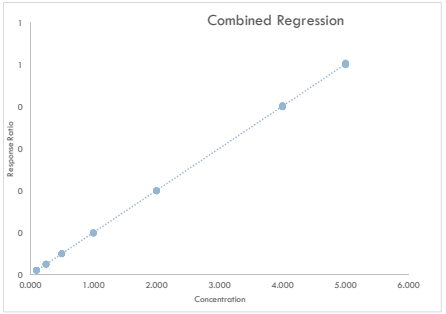
Intercept	8.4885E-06
Slope	0.099995376
r^2	0.999971066



Intercept	2.86487E-05
Slope	0.099984394
r^2	0.99999744



Intercept	-3.54059E-05
Slope	0.100011505
r^2	0.999988387



Intercept	1.15302E-05
Slope	0.099993719
r^2	0.9999874

Cnom	x	y	w (1/x)	wxy	wx	wy	wx^2	wy^2	Weighted Result
0.01	0.1	0.0102	1	0.001020000	0.1	0.010200000	0.01	0.000104040	0.010182135
0.025	0.25	0.0248	0.4	0.002480000	0.1	0.009920000	0.03	0.000246016	0.024782436
0.05	0.5	0.0498	0.2	0.004980000	0.1	0.009960000	0.05	0.000496008	0.049782952
0.1	1	0.0994	0.1	0.009940000	0.1	0.009940000	0.1	0.000988036	0.099383976
0.2	2	0.1999	0.05	0.019990000	0.1	0.009995000	0.2	0.001998001	0.199886051
0.4	4	0.3992	0.025	0.039920000	0.1	0.009980000	0.4	0.003984016	0.399190165
0.5	5	0.5018	0.02	0.050180000	0.1	0.010036000	0.5	0.005036065	0.501792283
0.01	0.1	0.0102	1	0.001020000	0.1	0.010200000	0.01	0.000104040	0.010164251
0.025	0.25	0.0249	0.4	0.002490000	0.1	0.009960000	0.03	0.000248004	0.024867282
0.05	0.5	0.0500	0.2	0.005000000	0.1	0.010000000	0.05	0.000500000	0.049972458
0.1	1	0.0989	0.1	0.009890000	0.1	0.009890000	0.1	0.000978121	0.098882541
0.2	2	0.1994	0.05	0.019940000	0.1	0.009970000	0.2	0.001988018	0.199403264
0.4	4	0.4003	0.025	0.040030000	0.1	0.010007500	0.4	0.004006002	0.400344689
0.5	5	0.5013	0.02	0.050130000	0.1	0.010026000	0.5	0.005026034	0.501314693
0.01	0.1	0.0102	1	0.001020000	0.1	0.010200000	0.01	0.000104040	0.010191983
0.025	0.25	0.0250	0.4	0.002500000	0.1	0.010000000	0.03	0.000250000	0.024992667
0.05	0.5	0.0498	0.2	0.004980000	0.1	0.009960000	0.05	0.000496008	0.049793814
0.1	1	0.0987	0.1	0.009870000	0.1	0.009870000	0.1	0.000974169	0.098696075
0.2	2	0.1987	0.05	0.019870000	0.1	0.009935000	0.2	0.001974085	0.198700700
0.4	4	0.3990	0.025	0.039900000	0.1	0.009975000	0.4	0.003980025	0.399009962
0.5	5	0.5036	0.02	0.050360000	0.1	0.010072000	0.5	0.005072259	0.503649952
0.01	0.1	0.0101	1	0.001010000	0.1	0.010100000	0.01	0.000102010	0.010072923
0.025	0.25	0.0249	0.4	0.002490000	0.1	0.009960000	0.03	0.000248004	0.024875233
0.05	0.5	0.0499	0.2	0.004990000	0.1	0.009980000	0.05	0.000498002	0.049879136
0.1	1	0.1000	0.1	0.010000000	0.1	0.010000000	0.1	0.001000000	0.099986956
0.2	2	0.2001	0.05	0.020010000	0.1	0.010005000	0.2	0.002002001	0.200102580
0.4	4	0.3991	0.025	0.039910000	0.1	0.009977500	0.4	0.003982020	0.399133641
0.5	5	0.5009	0.02	0.050090000	0.1	0.010018000	0.5	0.005018016	0.500949531
0.01	0.1	0.0101	1	0.001010000	0.1	0.010100000	0.01	0.000102010	0.010134240
0.025	0.25	0.0248	0.4	0.002480000	0.1	0.009920000	0.03	0.000246016	0.024832549
0.05	0.5	0.0499	0.2	0.004990000	0.1	0.009980000	0.05	0.000498002	0.049929661
0.1	1	0.0994	0.1	0.009940000	0.1	0.009940000	0.1	0.000988036	0.099423967
0.2	2	0.1993	0.05	0.019930000	0.1	0.009965000	0.2	0.001986025	0.199312475
0.4	4	0.4023	0.025	0.040230000	0.1	0.010057500	0.4	0.004046132	0.402289122
0.5	5	0.4991	0.02	0.049910000	0.1	0.009982000	0.5	0.004982016	0.499077986

Validation Study 1

CALIBRATION CURVE COMPARE

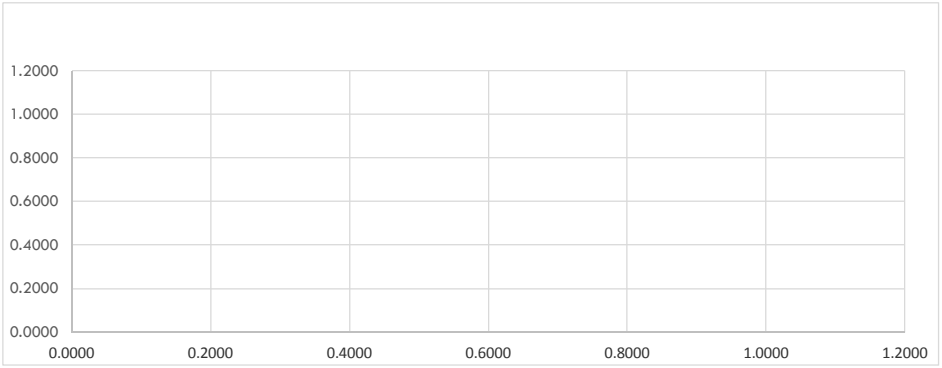
Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Sample	Full Cal	Case Cal

Slope	#DIV/0!
Standard error in slope	#VALUE!
Degrees Freedom	-1
Confidence Level	95%
Student t Value	#NUM!
Confidence Interval	#NUM!
Slope ± CI	#DIV/0!
Range	#DIV/0!
	#DIV/0!

Intercept	#DIV/0!
Standard error in intercept	#VALUE!
Degrees Freedom	-1
Confidence Level	95%
Student t Value	#NUM!
Confidence Interval	#NUM!
Intercept ± CI	#DIV/0!
Range	#DIV/0!
	#DIV/0!



Comments: Not performed; a 7-point calibration curve will be used for ethanol casework analysis.

Acceptance Criteria:

95% CI of slope includes 1
95% CI of intercept includes 0

Validation Study 2

LIMIT OF DETECTION

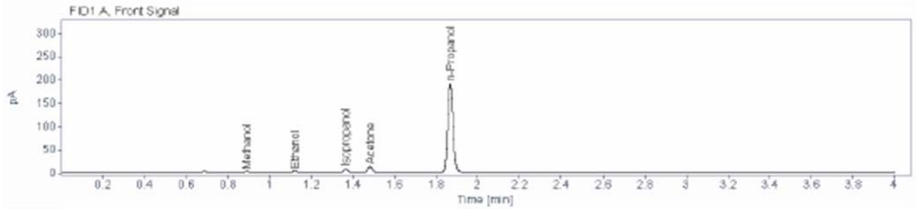
Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Run Date	Run Order	Negative	Signal:Noise		
Target Concentration (g/100 mL):		*0.0000*	*0.0075*	*0.0050*	*0.0025*
Run 1 05/05/16	1-1	0.000	188.541	496.090	308.241
	1-2		803.440	479.888	190.860
	1-3		716.606	656.125	218.862
	1-4		722.555	465.304	261.409
	1-5		774.753	519.552	245.661
	1-6		728.472	435.145	277.864
Run 2 05/06/16	2-1	0.000	242.953	489.432	262.089
	2-2		755.659	691.665	289.977
	2-3		777.807	528.783	246.681
	2-4		950.548	631.013	275.152
	2-5		782.043	497.154	285.154
	2-6		641.179	569.181	283.352
Run 3 05/09/16	3-1	0.000	215.904	548.796	217.939
	3-2		958.407	459.421	263.269
	3-3		608.570	516.654	230.451
	3-4		810.740	500.791	241.250
	3-5		818.205	494.880	238.361
	3-6		745.267	379.333	197.657
Mean Signal to Noise:		**0.000**	**680.092**	**519.956**	**251.902**

Sample chromatogram of LOD specimen:

Sample name: LOD - 0.0025A Description: Lot: 20160504-LOD25A Vial Number: 40
Instrument: Headspace 3 Acq. method: VOLATILES.M Injection date: 5/9/2016 8:11:37 PM



Established LOD: **0.0025 g/100 mL**
S:N of LOD: **251.902**

Comments: LOD samples were made using three sources of blank blood, W044616241086 (A), W0446162247837 (B), and W044616247845 (C). Theoretical target concentrations for LOD samples were 0.0025, 0.0050, and 0.0075 g/100 mL, using 0.125, 0.250, and 0.375 mL of 1000 µg/mL mixed volatile standard (lot FN09231404), respectively, diluted with each blood source to 5 mL.

Acceptance Criteria: Signal to Noise ≥ 3
Acceptable detection and identification criteria

Validation Study 3

LIMIT OF QUANTITATION

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Run Date	Run Order	LOQ
	Target Concentration (g/100 mL):	*0.0100*
Run 1 05/04/16	1-1	0.0101
	1-2	0.0102
	1-3	0.0102
Within Run	**Mean**	**0.0102**
	SD	**0.000058**
	%CV	**0.568%**
	% Bias	**1.67%**
Run 2 05/05/16	2-1	0.0101
	2-2	0.0102
	2-3	0.0101
Within Run	**Mean**	**0.0101**
	SD	**0.000058**
	%CV	**0.570%**
	% Bias	**1.33%**
Run 3 05/06/16	3-1	0.0101
	3-2	0.0101
	3-3	0.0101
Within Run	**Mean**	**0.0101**
	SD	**0.000000**
	%CV	**0.000%**
	% Bias	**1.00%**
Run 4 05/09/16	4-1	0.0098
	4-2	0.0100
	4-3	0.0099
Within Run	**Mean**	**0.0099**
	SD	**0.000100**
	%CV	**1.010%**
	% Bias	**-1.00%**
Run 5 05/11/16	5-1	0.0099
	5-2	0.0100
	5-3	0.0100
Within Run	**Mean**	**0.0100**
	SD	**0.000058**
	%CV	**0.579%**
	% Bias	**-0.33%**

Precision (%CV)	***Max Within-Run***	**1.01%**
	Between-Run	**0.38%**
% Bias		**0.53%**

Comments: Theoretical target concentration for LOQ (lot 20160503-LOQ) is 0.0100 g/100 mL, using 1.0 mL of 1000 µg/mL mixed volatile standard (lot FN09231404) diluted with drug-free pooled blood to 10 mL.

Acceptance Criteria:

Bias: ≤ ± 10%
Within-Run Imprecision: CV ≤ ± 10%
Between-Run Imprecision: CV ≤ ± 10%

Validation Study 4

BIAS AND PRECISION

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Run Date	Run Order	LMQC	MQC1	MQC2	HEQC
	Target Concentration (g/100 mL):	*0.0192*	*0.0789*	*0.1477*	*0.4000*
Run 1	1-1	0.0201	0.0785	0.1488	0.4053
05/04/16	1-2	0.0202	0.0787	0.1484	0.4040
	1-3	0.0202	0.0792	0.1476	0.4043
Within Run	**Mean**	**0.0202**	**0.0788**	**0.1483**	**0.4045**
	SD	**0.000058**	**0.000361**	**0.000611**	**0.000681**
	%CV	**0.286%**	**0.458%**	**0.412%**	**0.168%**
	% Bias	**5.03%**	**-0.13%**	**0.38%**	**1.13%**
Run 2	2-1	0.0201	0.0794	0.1476	0.4046
05/05/16	2-2	0.0201	0.0786	0.1480	0.4021
	2-3	0.0203	0.0789	0.1484	0.4018
Within Run	**Mean**	**0.0202**	**0.0790**	**0.1480**	**0.4028**
	SD	**0.000115**	**0.000404**	**0.000400**	**0.001537**
	%CV	**0.573%**	**0.512%**	**0.270%**	**0.382%**
	% Bias	**5.03%**	**0.08%**	**0.20%**	**0.71%**
Run 3	3-1	0.0201	0.0790	0.1474	0.4042
05/06/16	3-2	0.0202	0.0788	0.1477	0.4017
	3-3	0.0202	0.0788	0.1476	0.4018
Within Run	**Mean**	**0.0202**	**0.0789**	**0.1476**	**0.4026**
	SD	**0.000058**	**0.000115**	**0.000153**	**0.001415**
	%CV	**0.286%**	**0.146%**	**0.104%**	**0.352%**
	% Bias	**5.03%**	**-0.04%**	**-0.09%**	**0.64%**
Run 4	4-1	0.0199	0.0785	0.1472	0.4039
05/09/16	4-2	0.0200	0.0786	0.1479	0.4023
	4-3	0.0200	0.0789	0.1478	0.4029
Within Run	**Mean**	**0.0200**	**0.0787**	**0.1476**	**0.4030**
	SD	**0.000058**	**0.000208**	**0.000379**	**0.000808**
	%CV	**0.289%**	**0.265%**	**0.256%**	**0.201%**
	% Bias	**3.99%**	**-0.30%**	**-0.05%**	**0.76%**
Run 5	5-1	0.0200	0.0792	0.1475	0.4025
05/11/16	5-2	0.0201	0.0788	0.1468	0.4018
	5-3	0.0201	0.0795	0.1465	0.4035
Within Run	**Mean**	**0.0201**	**0.0792**	**0.1469**	**0.4026**
	SD	**0.000058**	**0.000351**	**0.000513**	**0.000854**
	%CV	**0.288%**	**0.444%**	**0.349%**	**0.212%**
	% Bias	**4.51%**	**0.34%**	**-0.52%**	**0.65%**

Mean		**0.0201**	**0.0789**	**0.1477**	**0.4031**
SD		**0.00010**	**0.00031**	**0.00060**	**0.00121**
Precision	***Max Within-Run***	**0.57%**	**0.51%**	**0.41%**	**0.38%**
(%CV)	***Between-Run***	**0.51%**	**0.40%**	**0.40%**	**0.30%**
% Bias		**4.72%**	**-0.01%**	**-0.01%**	**0.78%**

Comments: Theoretical target concentration for LMQC (lot 20160503-LMQC) is 0.0192 g/100 mL, using 1.2 mL 4000 µg/mL mixed volatile standard (lot FN01301503) diluted with deionized water to 25 mL. Target concentrations for MQC1 (lot 1304030) and MQC2 (lot 1407168) were established during this validation. Consequently, the bias for MQC1 and MQC2 could have been underestimated. However, as LMQC uses a nominal concentration as the target and still shows acceptable bias, performance of this method is deemed fit for purpose.

Acceptance Criteria:

Bias:
≤ ± 5% if target concentration is >0.05 g/100 mL
≤ ± 10% if target concentration is ≤0.05 g/100 mL
Within-Run Imprecision: CV ≤ ± 10%
Between-Run Imprecision: CV ≤ ± 10%

Validation Study 5

CARRYOVER

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Low Calibrator Target: 0.010 g/100 mL

Sample order	Results					
	Target Concentration (g/100 mL)	Day 1	Day 2	Day 3	Day 4	Day 5
Date		05/04/16	05/05/16	05/06/16	05/09/16	05/11/16
High Calibrator	0.5000	0.5018	0.5013	0.5036	0.5009	0.4991
Negative	0.0	0.00	0.00	0.00	0.00	0.00
% of Low Calibrator Level		**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**
					Maximum Carryover:	**0.0%**

Comments: Carryover evaluated by following the highest calibrator with a negative.

Acceptance Criteria: **Quantitative result ≤ 25% of LOQ**

Validation Study 6

MATRIX & IS INTERFERENCE

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

	LOQ Response
Run 1	16.4695
Run 2	16.4190
Run 3	16.9181
Run 4	16.9433
Run 5	17.0932
Average	16.7686

Identification	Peak Response	Comment
Blank Matrix (NO IS) 1	0	Pass
Blank Matrix (NO IS) 2	0	Pass
Blank Matrix (NO IS) 3	0	Pass
Blank Matrix (NO IS) 4	0	Pass
Blank Matrix (NO IS) 5	0	Pass
Blank Matrix (NO IS) 6	0	Pass
Blank Matrix (NO IS) 7	0	Pass
Blank Matrix (NO IS) 8	0	Pass
Blank Matrix (NO IS) 9	0	Pass
Blank Matrix (NO IS) 10	0	Pass
Blank Matrix (with IS) 1	0	Pass
Blank Matrix (with IS) 2	0	Pass
Blank Matrix (with IS) 3	0	Pass
Blank Matrix (with IS) 4	0	Pass
Blank Matrix (with IS) 5	0	Pass

Matrix Interference: None observed.
IS Interference: None observed.
Comments:

Acceptance Criteria: **Response of blank matrix ≤ 10% of the average response of LOQ**

Validation Study 6

EXOGENOUS SUBSTANCE INTERFERENCE

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Target Concentration: 0.0201 g/100 mL
Control Acceptance (%): 10

Sample #	Identification	Compound	Amount Added	Calculated Control Concentration	Comment
1	Ethyl Acetate	Ethyl Acetate	~0.2 g/100 mL	0.0217	Pass
2	Toluene	Toluene	~0.2 g/100 mL	0.0201	Pass
3	VWR® Laboratory Marker	Proprietary Component 1	Unable to Measure	0.0200	Pass
4		Proprietary Component 2	Unable to Measure		
5		Proprietary Solvent 1	Unable to Measure		
6		Proprietary Solvent 2	Unable to Measure		
7	Sharpie® Permanent Marker	n-Propanol	Unable to Measure	0.0047	Pass*
8		n-Butanol	Unable to Measure		
9		Diacetone Alcohol	Unable to Measure		
10		Dyes	Unable to Measure		

Conclusions: No compounds tested caused significant interference with the analyte of interest.

Comments: Toluene and ethyl acetate: mixed 500 µL of each reagent with 500 µL of deionized water, LMQC (lot 20160503-LMQC) and internal standard (20160429-I) were added to a headspace vial, 5 µL of either toluene or ethyl acetate diluted solution immediately added, and vials capped. Sharpie marker: the bottom of a headspace vial was mostly marked with Sharpie marker, LMQC and internal standard added immediately afterward, and vial capped. VWR marker: a small piece of Kim Wipe was extensively marked with VWR marker, added to a headspace vial, LMQC and internal standard added immediately afterward, and vial capped. *The Sharpie® Permanent Marker contained n-propanol, which more than quadrupled the internal standard signal and thus diminished the calculated value of the analyte of interest.

Acceptance Criteria: Concentration of analytes of interest meet acceptance criteria of the method.

Validation Study 7

DILUTION OF SAMPLES

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Run Date	Run Order	MQC2 Diluted 2x	MQC2 Calculated	% of Target	10% Ethanol Diluted 20x	Ethanol 1:20 Calculated	% of Target	10% Ethanol Diluted 50x	Ethanol 1:50 Calculated	% of Target	10% Ethanol Diluted 100x	Ethanol 1:100 Calculated	% of Target
Target Concentration (g/100 mL):			*0.1477*			*7.890*			*7.890*			*7.890*	
Run 1	1-1	0.0735	0.1470	-0.46	0.3720	7.4400	-5.70	0.1461	7.3050	-7.41	0.0707	7.0700	-10.39
	1-2	0.0736	0.1472	-0.33	0.3749	7.4980	-4.97	0.1457	7.2850	-7.67	0.0713	7.1300	-9.63
05/04/16	1-3	0.0736	0.1472	-0.33	0.3741	7.4820	-5.17	0.1475	7.3750	-6.53	0.0714	7.1400	-9.51
Run 2	2-1	0.0739	0.1478	0.08	0.3670	7.3400	-6.97	0.1475	7.3750	-6.53	0.0707	7.0700	-10.39
	2-2	0.0744	0.1488	0.76	0.3706	7.4120	-6.06	0.1479	7.3950	-6.27	0.0714	7.1400	-9.51
05/05/16	2-3	0.0738	0.1476	-0.05	0.3714	7.4280	-5.86	0.1491	7.4550	-5.51	0.0718	7.1800	-9.00
Run 3	3-1	0.0733	0.1466	-0.73	0.3676	7.3520	-6.82	0.1455	7.2750	-7.79	0.0712	7.1200	-9.76
	3-2	0.0734	0.1468	-0.60	0.3678	7.3560	-6.77	0.1456	7.2800	-7.73	0.0718	7.1800	-9.00
05/06/16	3-3	0.0738	0.1476	-0.05	0.3685	7.3700	-6.59	0.1467	7.3350	-7.03	0.0716	7.1600	-9.25
Run 4	4-1	0.0738	0.1476	-0.05	0.3677	7.3540	-6.79	0.1431	7.1550	-9.32	0.0710	7.1000	-10.01
	4-2	0.0735	0.1470	-0.46	0.3680	7.3600	-6.72	0.1426	7.1300	-9.63	0.0717	7.1700	-9.13
05/09/16	4-3	0.0738	0.1476	-0.05	0.3697	7.3940	-6.29	0.1436	7.1800	-9.00	0.0712	7.1200	-9.76
Run 5	5-1	0.0743	0.1486	0.62	0.3743	7.4860	-5.12	0.1449	7.2450	-8.17	0.0728	7.2800	-7.73
	5-2	0.0741	0.1482	0.35	0.3754	7.5080	-4.84	0.1454	7.2700	-7.86	0.0730	7.3000	-7.48
05/11/16	5-3	0.0744	0.1488	0.76	0.3775	7.5500	-4.31	0.1454	7.2700	-7.86	0.0732	7.3200	-7.22

Comments: The target concentration for MQC2 (lot 1407168) is based on the mean value obtained from the bias and precision study. The target concentration for the remaining dilutions is the concentration of the ethanol standard (lot 097K5012V; converted 10% v/v to w/v using the density of ethanol to obtain 7.890 g/100 mL). Analytical results were multiplied by the dilution factors to give the calculated value and compared to the target concentration. Case samples can be diluted by 1:2 prior to analysis. The higher dilution factors of 1:20, 1:50, and 1:100 will underestimate the ethanol concentration by up to approximately 7%, 10%, and 11%, respectively. If used, this method limitation will be disclosed on the test report.

Acceptance Criteria:
≤ ± 5% if target concentration is >0.05 g/100 mL
≤ ± 10% if target concentration is ≤0.05 g/100 mL

Validation Study 8

IN-PROCESS STABILITY

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Length of Time Delay (Unpunctured):

Acceptable Limit (%):

Run date	Sample I.D.	Results	Mean	Acceptable Range		Pass/Unstable
				Low	High	
			#DIV/0!			
			#DIV/0!			

Overall Acceptance:

Comments: Study not performed; it is not appropriate to break for extended periods during sample preparation.

Acceptance Criteria:

≤ ± 5% if target concentration is >0.05 g/100 mL
≤ ± 10% if target concentration is ≤0.05 g/100 mL

Validation Study 8

AUTOSAMPLER STABILITY

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Length of Time Delay (Unpunctured): 72 hours

Acceptable Limit (%): 5 if > 0.05 g/100 mL
Acceptable Limit (%): 10 if ≤ 0.05 g/100 mL

Run Date	Sample I.D.	Results	Mean	Bias & Precision Mean from 05/09/16	Acceptable Range		Pass/Unstable
					Low	High	
5/10/16	LMQC - 1	0.0204	**0.0204**	0.0200	0.0180	0.0220	Pass
	LMQC - 2	0.0202					
	LMQC - 3	0.0205					
	MQC1 - 1	0.0809	**0.0803**	0.0787	0.0747	0.0826	Pass
	MQC1 - 2	0.0800					
	MQC1 - 3	0.0801					
	MQC2 - 1	0.1482	**0.1495**	0.1476	0.1403	0.1550	Pass
	MQC2 - 2	0.1500					
	MQC2 - 3	0.1503					
	HEQC - 1	0.4039	**0.4054**	0.4030	0.3829	0.4232	Pass
	HEQC - 2	0.4061					
	HEQC - 3	0.4061					
5/11/16	LMQC - 1	0.0208	**0.0207**	0.0200	0.0180	0.0220	Pass
	LMQC - 2	0.0207					
	LMQC - 3	0.0205					
	MQC1 - 1	0.0816	**0.0814**	0.0787	0.0747	0.0826	Pass
	MQC1 - 2	0.0813					
	MQC1 - 3	0.0813					
	MQC2 - 1	0.1522	**0.1520**	0.1476	0.1403	0.1550	Pass
	MQC2 - 2	0.1520					
	MQC2 - 3	0.1519					
	HEQC - 1	0.4059	**0.4055**	0.4030	0.3829	0.4232	Pass
	HEQC - 2	0.4042					
	HEQC - 3	0.4065					
5/12/16	LMQC - 1	0.0206	**0.0205**	0.0200	0.0180	0.0220	Pass
	LMQC - 2	0.0204					
	LMQC - 3	0.0205					
	MQC1 - 1	0.0800	**0.0800**	0.0787	0.0747	0.0826	Pass
	MQC1 - 2	0.0804					
	MQC1 - 3	0.0795					
	MQC2 - 1	0.1502	**0.1507**	0.1476	0.1403	0.1550	Pass
	MQC2 - 2	0.1513					
	MQC2 - 3	0.1507					
	HEQC - 1	0.3948	**0.3974**	0.4030	0.3829	0.4232	Pass
	HEQC - 2	0.3991					
	HEQC - 3	0.3983					

Results: Samples on the autosampler may be analyzed for ethanol up to 72 hours after preparation.
Comments:

Acceptance Criteria:
≤ ± 5% if target concentration is >0.05 g/100 mL
≤ ± 10% if target concentration is ≤0.05 g/100 mL

Validation Study 8

STORAGE STABILITY

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

	LQC Day 0	HQC Day 0
Mean		
Acceptance %:	**20**	

Storage Conditions	Sample ID	Day 1	Day 2	Day 7	Day 14	Day 30
Room Temperature						
Room Temperature w/ Light Protection						
Refrigerated (2 to 8°C)						
Frozen (-5 to -20°C)						

Summary:

	Days
Room Temperature (RT):	
RT Light Protected:	
Refrigerated:	
Frozen:	

Comments: Study not performed; the stability of ethanol in storage conditions is well documented in the literature.

Acceptance Criteria: **≤ ± 5% if target concentration is >0.05 g/100 mL**
≤ ± 10% if target concentration is ≤0.05 g/100 mL

Validation Study 7

MATRIX MATCHING

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Matrix: ***Blood***

Run Date	Run Order	Low Control	Medium Control	High Control
Target Concentration (g/100 mL):				
Run 1	1-1			
	1-2			
	1-3			
Within Run	**Mean**			
	SD			
	%CV			
	% Bias			
Run 2	2-1			
	2-2			
	2-3			
Within Run	**Mean**			
	SD			
	%CV			
	% Bias			
Run 3	3-1			
	3-2			
	3-3			
Within Run	**Mean**			
	SD			
	%CV			
	% Bias			
Mean		#DIV/0!	#DIV/0!	#DIV/0!
SD		#DIV/0!	#DIV/0!	#DIV/0!
Precision (%CV)	***Max Within-Run***	0.00%	0.00%	0.00%
	Between-Run			
% Bias				

Matrix: ***Water***

Run Date	Run Order	Low Control	Medium Control	High Control
Target Concentration (g/100 mL):				
Run 1	1-1			
	1-2			
	1-3			
Within Run	**Mean**			
	SD			
	%CV			
	% Bias			
Run 2	2-1			
	2-2			
	2-3			
Within Run	**Mean**			
	SD			
	%CV			
	% Bias			
Run 3	3-1			
	3-2			
	3-3			
Within Run	**Mean**			
	SD			
	%CV			
	% Bias			
Mean		#DIV/0!	#DIV/0!	#DIV/0!
SD		#DIV/0!	#DIV/0!	#DIV/0!
Precision (%CV)	***Max Within-Run***	0.00%	0.00%	0.00%
	Between-Run			
% Bias				

Conclusions:		***Low***	***Medium***	***High***
Max %Bias	***Blood***			
Max %CV				
Max %Bias	***Water***			
Max %CV				

Comments: Study not performed; all samples were diluted 10x before analysis.

Acceptance Criteria:

≤ ± 5% if target concentration is >0.05 g/100 mL
≤ ± 10% if target concentration is ≤0.05 g/100 mL
% CV ≤ ± 10%

Additional Study — ACETALDEHYDE

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

Run Date	Sample I.D.	Retention Time (min)	Mean (min)
05/06/16	Low	0.941	**0.941**
	Low	0.941	
	Low	0.941	
	Med	0.940	**0.940**
	Med	0.940	
	Med	0.940	
	High	0.940	**0.940**
	High	0.940	
	High	0.940	

Average Retention Time (min): **0.940**

Comments: The retention time of acetaldehyde was determined by diluting reagent grade acetaldehyde (lot 00162EH) with deionized water at three concentrations (approximately 0.02 g/100 mL (low), 0.10 g/100 mL (med), and 0.35 g/100 mL (high)), aliquoting with IS, and recording the retention times of the resulting peaks. The presence of a peak at both 0.940 minutes on FID1 and at 0.797 minutes on FID2 indicates the presence of acetaldehyde.

Acceptance Criteria: **(Not applicable)**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: ***Ethanol***
Units: ***g/100 mL***
Instrument: ***Headspace 3 - FID1***

Analyst: ***Matthew A. Cheney, Ph.D.***
Study Dates: ***05/04/16 to 05/12/16***
Primary Matrix: ***Whole Blood***

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason

Deviations from SOP:

Other Observations:

The temperatures for blank blood lots 247837 and 247845, used to prepare the LOQ and LOD controls, were not documented at the donation site. Lot 247837 was also used for the matrix interference study. Within batches 20160505_MAC and 20160506_MAC, the lot numbers for all nine LOD samples began with "20150504" but should have been "20160504" to reflect the date of preparation.

Comments:

Recommended Maximum Run Length (# Unknown Samples): 60

Conclusion:

This method is fit for casework analysis of ethanol.