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Date: 08/13/15

Subject: Validation of Agilent GC (serial numbers US14173023 and CN14160045)

Background:

A new Agilent GC consisting of an Agilent 7890B GC (#US14173023) and a 7697A headspace sampler (CN14160045) was installed in the Toxicology Lab in July 2014. The intended purpose for this instrument was the analysis of samples for blood alcohol concentration using headspace analysis. The proposed validation plan was approved in December 2014 (see Appendix 1). Validation testing took place from January 2015 through April 2015. The test results are detailed in this document.

Deviations from validation plan:

During the validation process, adjustments were made to the work proposed in the validation plan. None of the adjustments changed the acceptance criteria.

The validation plan proposed a GC cycle time of 3.5 minutes. The GC cycle time was extended to 4 minutes.

Evaluation of the calibration model was expanded from five runs on five different days to ten runs on five different days.

Evaluation of the detector response over the range of 0.01 g/dL to 0.50 g/dL was expanded from three runs over three days to five runs over five days.

The level at which bias and precision was evaluated was expanded from 0.04 g/dL, 0.08 g/dL, 0.15 g/dL, 0.2 g/dL and 0.4 g/dL to include 0.3 g/dL. A 0.3 g/dL whole blood control was ordered from a vendor that the Lab had not previously used. The Medichem whole blood control, when tested in the lab, did not meet Lab requirements that the measured value be within 3% of the manufacturer's value. The control was run throughout the validation study; however, the results are not included in the evaluation of the instrument. An aqueous control and whole blood control around the 0.3 g/dL level from other vendors were included in evaluation of the instrument's performance.

The evaluation of matrix interference was expanded to include blood drawn from ten different individuals.

Evaluation of determining the limit of detection was expanded to include evaluating the y-intercept and slope of the calibration line from fourteen different calibrations.

Method development:

The main analytical method developed was named ethanol quant. In addition, two other methods were developed for use with a six-point calibration curve from 0.02 g/dL to 0/40 g/dL and from 0.01 g/dL to 0.50 g/dL. These two methods were called ethanol quant 6 pt cal and ethanol quant 01 to 50 cal, respectively. These two methods had the same instrumental parameters as ethanol quant. The ethanol quant method parameters are detailed in the following table.

Table 1. Operating Paramaters

	7890B GC
GC oven	40° C
GC run time	2.8 minutes
GC cycle time	4 minutes
Carrier gas	hydrogen
Front inlet temperature	110° C
Pressure	10 PSI
Septum purge flow	3 ml/min
Split ratio	10:1
Column A	DB-ALC1 30m x 320 µm X 1.8 µm
Column B	DB-ALC2 30m x 320 µm X 1.8 µm
FID temperature	300° C
FID hydrogen flow	30 ml/min
FID air flow	400 ml/min
FID nitrogen flow	25 ml/min
	7697A headspace
Vial pressurization gas	nitrogen
Loop size	1 ml
Oven temperature	60° C
Loop temperature	60° C
Transfer line temperature	90° C
Vial equilibration	22 minutes
Injection duration	0.5 minutes
GC cycle time	4.0 minutes
Vial fill pressure	15 psi
Loop ramp rate	30 psi/min
Loop final pressure	1.5 psi
Loop equilibration time	0.05
Post injection purge	100 ml/min for 1 min

All samples analyzed as part of the validation were prepared using pipette diluter MD91JC4962 and internal standard lot 102214.

Calibration Model

The applicability of a linear calibration model was tested using data from ten runs spread over five days. The following calibration concentrations were used in each run: 0.02 g/dL, 0.10 g/dL, 0.15 g/dL, 0.20 g/dL, 0.30 g/dL, and 0.40 g/dL. All calibrators were aqueous solutions purchased from Cerilliant (see appendix 2 for certificates of analysis). The correlation coefficient (r) and coefficient of determination (r^2) for each calibration are shown in Table 2.

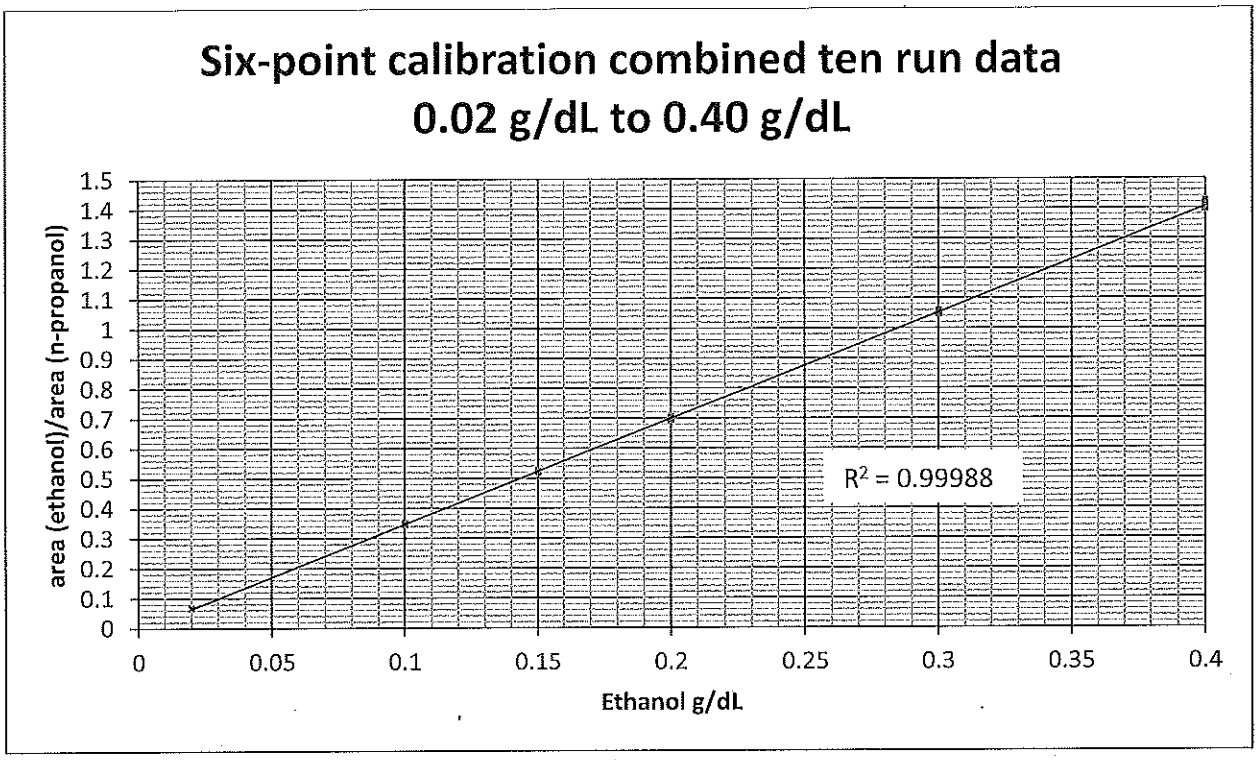
Table 2. Correlation coefficients and coefficients of determination for calibration runs

run	Correlation coefficient (r)	Coefficient of determination (r^2)	sequence
Run 1 day 1	0.99999	0.99999	Cal model run day1
Run 2 day 1	0.99999	0.99999	Cal model run day1b
Run 1 day 2	0.99994	0.99988	Cal model run day2
Run 2 day 2	0.99998	0.99996	Cal model run day2b
Run 1 day 3	0.99999	0.99997	Cal model run day3
Run 2 day 3	0.99997	0.99995	Cal model run day3b
Run 1 day 4	1.00000	0.99999	Cal model run day4
Run 2 day 4	1.00000	0.99999	Cal model run day4b
Run 1 day 5	0.99996	0.99991	Cal model run day5
Run 2 day 5	0.99998	0.99997	Cal model run day5b

The r^2 values obtained in each of the ten calibrations exceeds the required 0.995.

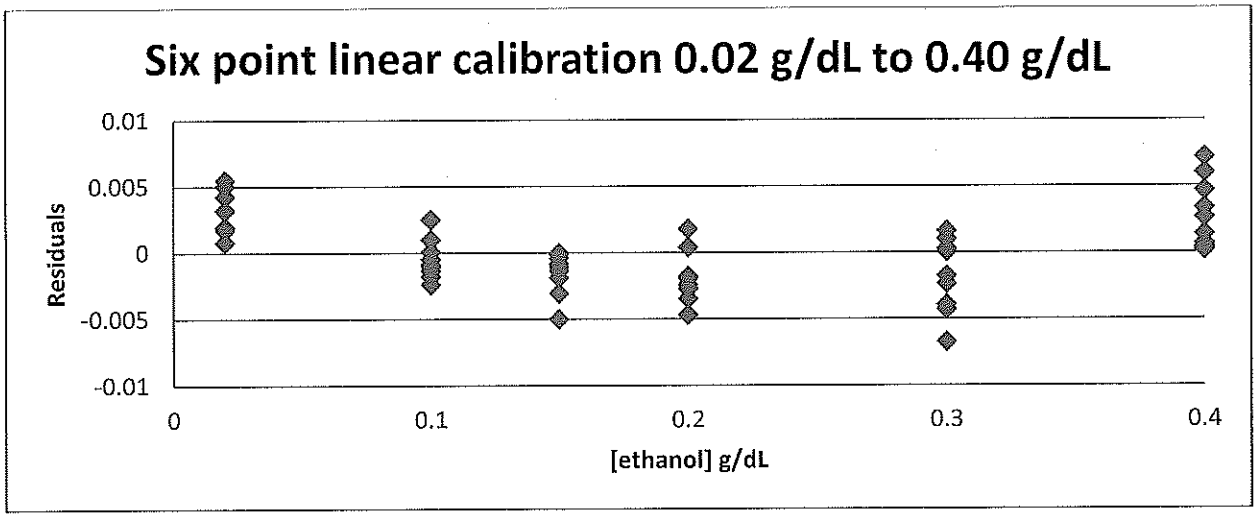
All of the data generated from the ten calibration runs were plotted together (see figure 1). The correlation between the detector response and ethanol concentration was 0.99994. **The r^2 value 0.99988 is greater than 0.995.**

Figure 1. Data from all calibrations runs plotted together.



In addition to looking at the r^2 values for each run, the residuals were plotted for each concentration (see figure 2).

Figure2. Residuals plotted at each concentration for the ten calibrations.



- Linearity of Detector Response 0.01 g/dL to 0.50 g/dL:

Outside of establishing the calibration model, the linearity of detector response with change in ethanol concentration was also evaluated over the range of 0.01 g/dL to 0.50 g/dL. Five runs over five different days were conducted using the following calibration concentrations: 0.01 g/dL, 0.10 g/dL, 0.20 g/dL, 0.30 g/dL, 0.40 g/dL, and 0.50 g/dL. All calibrators were aqueous solutions purchased from Cerilliant (see appendix 2 for certificates of analysis). The correlation coefficient (r) and coefficient of determination (r^2) for each calibration are shown in Table 3.

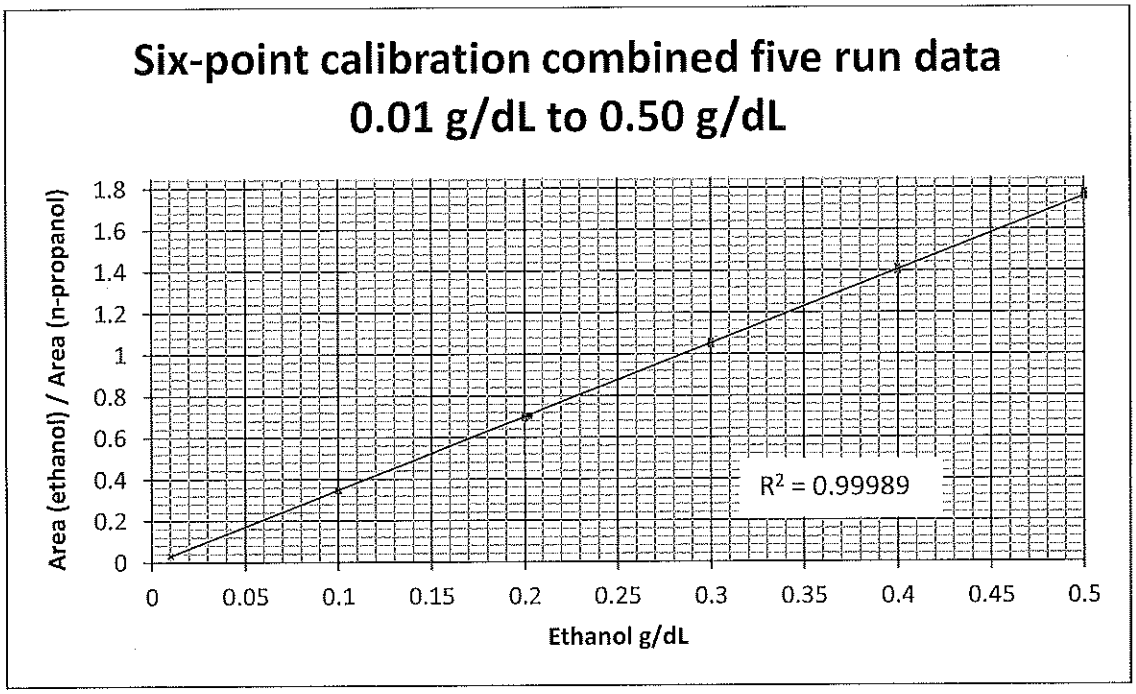
Table 3. Correlation coefficients and coefficients of determination for calibration runs

run	Correlation coefficient (r)	Coefficient of determination (r^2)	sequence
Day 1	0.99999	0.99999	cal 01 to 50 day1
Day 2	0.99999	0.99998	cal 01 to 50 day2
Day 3	0.99999	0.99998	cal 01 to 50 day3
Day 4	0.99999	0.99998	cal 01 to 50 day4
Day 5	0.99999	0.99998	cal 01 to 50 day5

The r^2 values obtained in each of the five calibrations exceeds the required 0.995.

All of the data generated from the five calibration runs was plotted together (see figure 3). The correlation between the detector response and ethanol concentration was 0.99994. **The r^2 value 0.99989 is greater than 0.995.**

Figure 3. Data from all calibrations runs plotted together.



A linear model will be used for the rest of the validation runs. The following four calibration levels will be used: 0.02 g/dL, 0.10 g/dL, 0.20 g/dL, and 0.40 g/dL.

Bias:

Bias was evaluated using runs on five different days with two different analysts. Each run contained three samples for each reference material analyzed. The following aqueous reference materials were analyzed: Lipomed 0.04 g/dL, Lipomed 0.08 g/dL, Lipomed 0.15 g/dL, Cerilliant 0.30 g/dL, and Lipomed 0.40 g/dL. The following whole blood reference materials were analyzed: CliniQA 0.077 g/dL, ACQ Science 0.2002 g/dL, and CliniQA 0.292 g/dL.

Bias was calculated as follows:

$$Bias\ (\%)\ at\ Concentration_x = \left[\frac{Grand\ Mean\ of\ Calculated\ Concentration_x - Nominal\ Concentration_x}{Nominal\ Concentration_x}\right] x100$$

Table 4. Bias calculated for each control.

	Lipomed	CliniQA	Lipomed	Lipomed	ACQ	CliniQA	Cerilliant	Lipomed
Run 1	0.0391	0.0795	0.0791	0.1487	0.2043	0.2954	0.3036	0.4037
	0.0395	0.0780	0.0796	0.1501	0.2031	0.2966	0.3040	0.4029
	0.0392	0.0788	0.0802	0.1494	0.2032	0.2938	0.3028	0.4001
Run 2	0.0382	0.0768	0.0776	0.1473	0.2008	0.2950	0.3016	0.4012
	0.0382	0.0774	0.0785	0.1478	0.2010	0.2978	0.3008	0.3995
	0.0383	0.0769	0.0789	0.1507	0.2037	0.2959	0.3006	0.4011
Run 3	0.0405	0.0783	0.0796	0.1464	0.2000	0.2941	0.2961	0.3939
	0.0407	0.0786	0.0798	0.1475	0.2012	0.2934	0.2976	0.3955
	0.0410	0.0782	0.0800	0.1480	0.2001	0.2949	0.2970	0.3933
Run 4	0.0396	0.0781	0.0794	0.1482	0.2019	0.2949	0.3007	0.4007
	0.0396	0.0780	0.0798	0.1490	0.2018	0.2994	0.3028	0.4002
	0.0395	0.0782	0.0801	0.1499	0.2020	0.2956	0.3022	0.4001
Run 5	0.0390	0.0781	0.0804	0.1488	0.2025	0.2950	0.3041	0.4034
	0.0393	0.0779	0.0796	0.1490	0.2025	0.2947	0.3056	0.4031
	0.0395	0.0773	0.0800	0.1516	0.2036	0.2960	0.3047	0.4020
nominal	**0.0400**	**0.0773**	**0.0800**	**0.1500**	**0.2002**	**0.292**	**0.3000**	**0.4000**
mean	**0.0394**	**0.0780**	**0.0795**	**0.1488**	**0.2021**	**0.2955**	**0.3016**	**0.4000**
Bias %	**-1.47**	**0.91**	**-0.62**	**-0.78**	**0.96**	**1.20**	**0.54**	**0.01**

The bias at each concentration was less than the criterion set in the validation plan of ±5%.

The following sequences apply to the bias section: 03Mar15 b, 05Mar15 b, 06Mar15, 09Mar15, 17Mar15, 18Mar15, 19Mar15, 20Mar15, and 23Mar15.

Precision:

Both within-run and between-run precision was was evaluated using runs on five different days with two different analysts. Each run contained three samples for each reference material analyzed. The

following aqueous reference materials were analyzed: Lipomed 0.04 g/dL, Lipomed 0.08 g/dL, Lipomed 0.15 g/dL, Cerilliant 0.30 g/dL, and Lipomed 0.40 g/dL. The following whole blood reference materials were analyzed: CliniQA 0.077 g/dL, ACQ Science 0.2002 g/dL, and CliniQA 0.292 g/dL.

- Within-run Precision

Within-run precision was calculated as follows:

$$Within-run\ CV\ (\%) = \frac{std\ deviation\ of\ a\ single\ run\ of\ samples}{mean\ calculated\ value\ of\ a\ single\ run\ of\ samples}\ x\ 100$$

Table 5. Within-run precision calculated for each control.

	Lipomed	CliniQA	Lipomed	Lipomed	ACQ	CliniQA	Cerilliant	Lipomed
Run 1	0.0391	0.0795	0.0791	0.1487	0.2043	0.2954	0.3036	0.4037
	0.0395	0.0780	0.0796	0.1501	0.2031	0.2966	0.3040	0.4029
	0.0392	0.0788	0.0802	0.1494	0.2032	0.2938	0.3028	0.4001
mean	**0.0393**	**0.0788**	**0.0796**	**0.1494**	**0.2035**	**0.2953**	**0.3034**	**0.4022**
Std dev	**0.00021**	**0.00075**	**0.00055**	**0.00070**	**0.00067**	**0.0014**	**0.00061**	**0.0019**
CV %	**0.53**	**0.95**	**0.69**	**0.47**	**0.33**	**0.48**	**0.20**	**0.47**
Run 2	0.0382	0.0768	0.0776	0.1473	0.2008	0.2950	0.3016	0.4012
	0.0382	0.0774	0.0785	0.1478	0.2010	0.2978	0.3008	0.3995
	0.0383	0.0769	0.0789	0.1507	0.2037	0.2959	0.3006	0.4011
mean	**0.0382**	**0.0770**	**0.0783**	**0.1486**	**0.2018**	**0.2962**	**0.3010**	**0.4006**
Std dev	**0.00006**	**0.00032**	**0.00067**	**0.0018**	**0.0016**	**0.0014**	**0.00053**	**0.00095**
CV %	**0.15**	**0.42**	**0.85**	**1.24**	**0.80**	**0.48**	**0.18**	**0.24**
Run 3	0.0405	0.0783	0.0796	0.1464	0.2000	0.2941	0.2961	0.3939
	0.0407	0.0786	0.0798	0.1475	0.2012	0.2934	0.2976	0.3955
	0.0410	0.0782	0.0800	0.1480	0.2001	0.2949	0.2970	0.3933
mean	**0.0407**	**0.0784**	**0.0798**	**0.1473**	**0.2004**	**0.2941**	**0.2969**	**0.3942**
Std dev	**0.00025**	**0.00021**	**0.00020**	**0.00082**	**0.00067**	**0.00075**	**0.00076**	**0.0011**
CV %	**0.62**	**0.26**	**0.25**	**0.56**	**0.33**	**0.26**	**0.25**	**0.29**
Run 4	0.0396	0.0781	0.0794	0.1482	0.2019	0.2949	0.3007	0.4007
	0.0396	0.0780	0.0798	0.1490	0.2018	0.2994	0.3028	0.4002
	0.0395	0.0782	0.0801	0.1499	0.2020	0.2956	0.3022	0.4001
Mean	**0.0396**	**0.0781**	**0.0798**	**0.1490**	**0.2019**	**0.2966**	**0.3019**	**0.4003**
Std dev	**0.00006**	**0.00010**	**0.00035**	**0.00085**	**0.00010**	**0.0024**	**0.0011**	**0.00032**
CV %	**0.14**	**0.13**	**0.44**	**0.57**	**0.05**	**0.82**	**0.36**	**0.08**
Run 5	0.0390	0.0781	0.0804	0.1488	0.2025	0.2950	0.3041	0.4034
	0.0393	0.0779	0.0796	0.1490	0.2025	0.2947	0.3056	0.4031
	0.0395	0.0773	0.0800	0.1516	0.2036	0.2960	0.3047	0.4020
mean	**0.0393**	**0.0778**	**0.0800**	**0.1498**	**0.2029**	**0.2952**	**0.3048**	**0.4028**
Std dev	**0.00025**	**0.00041**	**0.00040**	**0.0016**	**0.00064**	**0.00068**	**0.00076**	**0.00073**
CV %	**0.64**	**0.53**	**0.50**	**1.04**	**0.31**	**0.23**	**0.25**	**0.18**

The % CV for all runs at each concentration was less than the criterion set in the validation plan of ±10%.

- Between-run Precision

Between-run precision was calculated as follows:

$$Between-run\ CV\ (\%) = \frac{std\ deviation\ of\ grand\ mean\ for\ each\ concentration}{grand\ mean\ for\ each\ concentration}\ x\ 100$$

Table 6. Between-run precision calculated for each control.

	Lipomed	CliniQA	Lipomed	Lipomed	ACQ	CliniQA	Cerilliant	Lipomed
Run 1	0.0391	0.0795	0.0791	0.1487	0.2043	0.2854	0.3036	0.4037
	0.0395	0.0780	0.0796	0.1501	0.2031	0.2966	0.3040	0.4029
	0.0392	0.0788	0.0802	0.1494	0.2032	0.2938	0.3028	0.4001
Run 2	0.0382	0.0768	0.0776	0.1473	0.2008	0.2950	0.3016	0.4012
	0.0382	0.0774	0.0785	0.1478	0.2010	0.2978	0.3008	0.3995
	0.0383	0.0769	0.0789	0.1507	0.2037	0.2959	0.3006	0.4011
Run 3	0.0405	0.0783	0.0796	0.1464	0.2000	0.2941	0.2961	0.3939
	0.0407	0.0786	0.0798	0.1475	0.2012	0.2934	0.2976	0.3955
	0.0410	0.0782	0.0800	0.1480	0.2001	0.2949	0.2970	0.3933
Run 4	0.0396	0.0781	0.0794	0.1482	0.2019	0.2949	0.3007	0.4007
	0.0396	0.0780	0.0798	0.1490	0.2018	0.2994	0.3028	0.4002
	0.0395	0.0782	0.0801	0.1499	0.2020	0.2956	0.3022	0.4001
Run 5	0.0390	0.0781	0.0804	0.1488	0.2025	0.2950	0.3041	0.4034
	0.0393	0.0779	0.0796	0.1490	0.2025	0.2947	0.3056	0.4031
	0.0395	0.0773	0.0800	0.1516	0.2036	0.2960	0.3047	0.4020
Std dev	**0.00084**	**0.00070**	**0.00073**	**0.0014**	**0.0013**	**0.0015**	**0.0029**	**0.0033**
mean	**0.0394**	**0.0780**	**0.0795**	**0.1488**	**0.2021**	**0.2955**	**0.3016**	**0.4000**
CV %	**2.14**	**0.90**	**0.92**	**0.92**	**0.65**	**0.52**	**0.95**	**0.83**

The % CV for all runs at each concentration was less than the criterion set in the validation plan of ±10%.

The following sequences apply to the precision section: 03Mar15 b, 05Mar15 b, 06Mar15, 09Mar15, 17Mar15, 18Mar15, 19Mar 15, 20Mar15, and 23Mar15.

Carryover:

The potential for carryover was evaluated by running ethanol-free blood from an individual following a reference material with a 0.5 g/dL ethanol concentration. The runs containing the blank blood sample were the same five runs described above for the 0.01 to 0.5 calibration study. The blood sample was drawn into a gray-top vacutainer tube February 24, 2014 (sample 022414PAK). Ethanol was not detected in the blank blood samples in the five runs.

The potential for carryover was also evaluated by running aqueous blank samples following two different mixtures of volatile compounds as part of the interference studies. Mixture 1 was lot 021814VPV of the labs volatile mixture. This sample contained methanol, acetaldehyde ethanol, isopropanol, acetone and n-propanol. None of these compounds were detected in the blank following the volatile mixture sample. Mixture 2 was FN122210-01 from Cerilliant. This sample contained methanol, ethanol, isopropanol, acetone, and n-propanol. None of these compounds were detected in the blank following the volatile mixture sample.

The system appears to be free from carryover at least up to concentration of 0.5 g/dL.

The following sequences apply to the carryover section: cal 01 to 50 day 1, cal 01 to 50 day 2, cal 01 to 50 day 3, cal 01 to 50 day 4, cal 01 to 50 day 5, and interference 012615.

Interference Studies:

Blood from ten different individuals was collected in vacutainer tubes. Samples from this blood were analyzed on the instrument without the addition of internal standard. Nothing was detected in the chromatograms from the blood matrix of these individuals that would affect the measurement of n-propanol or ethanol using this instrument/method.

If a sample contains toluene, the toluene does not elute within the chromatogram run time for the sample. The toluene from a sample appears in the chromatogram two samples after the chromatogram for the sample containing toluene. To evaluate the possible interference of toluene with quantitation of ethanol in subsequent samples, two samples containing toluene were analyzed as a pair of duplicates followed by four samples of an aqueous 0.04 g/dL control. The peaks for toluene are not at the same times as ethanol or the internal standard and do not interfere with quantitation of ethanol. Table 7 shows the results samples from sequence interference 012615. The four control samples run immediately after the four calibrators and the four control samples run after the samples containing toluene have the same measured reportable concentration.

Sequence interference 012615 also contained 2 samples of volatile mixture 021814VPV and two samples of volatile mixture FN122210-01. For each of the chromatograms for these mixtures, there was baseline separation of the internal standard and of all of the components of the mixture. In addition one sample of each mixture was followed by four samples of an aqueous 0.04 g/dL control. The four control samples run immediately after the four calibrators and the four control samples run after the samples containing the volatile mixtures all have the same measured reportable concentration.

Table 7. Values obtained for 0.04 g/dL control under different conditions.

	After calibrators	After toluene samples	After mix 021814VPV	After mix FN122210-01
Sample 1	0.0394 g/dL	0.0397 g/dL	0.0396 g/dL	0.0397 g/dL
Sample 2	0.0395 g/dL	0.0398 g/dL	0.0400 g/dL	0.0398 g/dL
Sample 3	0.0395 g/dL	0.0394 g/dL	0.0396 g/dL	0.0400 g/dL
Sample 4	0.0393 g/dL	0.0400 g/dL	0.0398 g/dL	0.0398 g/dL
Mean value	**0.0394** g/dL	**0.0397** g/dL	**0.0397** g/dL	**0.0398** g/dL
Reported value	**0.039** g/dL	**0.039** g/dL	**0.039** g/dL	**0.039** g/dL

The presence of the volatiles tested does not interfere with the quantitation of ethanol.

Limit of detection/Limit of quantitation:

The limit of detection (an estimate of the lowest concentration of ethanol that can be reliably differentiated from a blank) was estimated as part of the validation. The limit of quantitation (an estimate of the lowest ethanol concentration that can be measured with acceptable bias and precision) was also estimated as part of the validation.

- **Limit of Detection**

The limit of detection was estimated using the linear calibration curves from fourteen runs calibrated using the following concentrations: 0.02 g/dL, 0.10 g/dL, 0/20 g/dL, and 0.40 g/dL. The estimation of the limit of detection was calculated as 3 times the standard deviation of the y-intercept divided by the average slope for the calibration line from fourteen runs.

Table 8. Slope and y-intercept data from fourteen calibrations.

Sequence name	y-intercept	slope
03Mar15b	-0.0034324	3.507108
05Mar15b	0.0008681	3.520183
06Mar15	-0.0117534	3.603896
09Mar15	-0.0045068	3.541089
23Mar15	-0.0028315	3.492029
17Mar15	-0.0049963	3.508168
27Mar15*	-0.0039789	3.505579
30Mar15*	-0.0042037	3.522523
31Mar15*	-0.0053841	3.513771
01Apr15*	-0.00241	3.486285
02Apr15*	-0.0035008	3.52138
18Mar15	-0.0034876	3.491913
19Mar15	-0.0036139	3.502202
20Mar15	-0.000432	3.478788
Standard deviation of y-intercept		0.00283166
Mean slope		3.513923
Estimated limit of detection		0.002418 g/dL

*Although 4 of these sequences have the same name as those used for the limit of quantitation evaluation in the validation, these five runs were outside of the validation and part of the runs incorporated into the uncertainty of measurement estimate.

The estimated limit of detection is 0.0024 g/dL (see table 8). **The limit of detection will be administratively set at 0.010 g/dL.**

- Limit of Quantitation

The limit of quantitation was evaluated using four batches run on 4 different days. Each batch contained three samples of aqueous controls at 0.01 g/dL and three at 0.02 g/dL.

Table 9. Analysis of 0.01 g/dL and 0.02 g/dL controls.

Sequence name	Measured g/dL	Measured g/dL
27 Mar 15	0.0104	0.0205
	0.0103	0.0205
	0.0104	0.0206
30Mar15	0.0112	0.0212
	0.0110	0.0212
	0.0112	0.0211
31Mar15	0.0106	0.0206
	0.0105	0.0206
	0.0106	0.0207
01Apr15	0.0109	0.0211
	0.0111	0.0209
	0.0110	0.0210
mean	0.01077	0.02083
Std dev	0.000334	0.000277
Bias	7.7%	4.2%
Between run CV	3.1%	1.3%
Within run 1 CV	0.6%	0.3%
Within run 2 CV	1.0%	0.3%
Within run 3 CV	0.5%	0.3%
Within run 4 CV	1.3%	0.6%

The % CV for all runs of the 0.01 g/dL control was less than the criterion set in the validation plan of ±10%. The bias was greater than the criterion set in the validation plan of ±5%. However, the absolute bias is less than 0.0008 g/dL.

The % CV for all runs of the 0.02 g/dL control was less than the criterion set in the validation plan of ±10%. The bias was less than the criterion set in the validation plan of ±5%. The limit of quantitation will be administratively set at the level of the lowest calibrator, 0.02 g/dL.

Recommendation:

The Agilent GC has passed its performance checks and should be authorized for casework.

P. Allan Kosecki

Kris Cano

APPENDIX ONE

Date: December 4, 2014

To: Kris Cano, Lab Manager

From: P. Allan Kosecki, Forensic Scientist

Re: Validation Plan for Headspace Blood Alcohol Determination Using Agilent Gas Chromatograph

---

Purpose/Scope

The purpose of this validation study is to determine if the Agilent GC (US14173023) with autosampler (CN14160045) is appropriate for use in making blood alcohol determinations in the Toxicology Section using the methods developed in this study. This study will produce objective evidence to demonstrate that the requirements set forth in this plan were or were not met. The methods and instrument will be operated in the controlled environment of the toxicology examination room.

Materials/Instrumentation

Agilent GC model 7890B with dual FID
Agilent autosampler model 7697A
Agilent DB-ALC1 30-meter column
Agilent DB-ALC2 30-meter column
Aqueous ethanol controls
Whole blood ethanol controls
20 ml headspace vials with caps
Blank whole blood
Volatiles mixture
Hamilton Diluter/Dispenser
Hydrogen generator
Nitrogen generator
Air generator
0.015% w/v n-propanol

Sample Preparation

Samples will be prepared for analysis per the procedures documented in the Blood Alcohol Analysis Procedures Manual.

Analytical Method

The analysis will be a headspace analysis using n-propanol as an internal standard. Hydrogen will be used as the carrier gas. Headspace vials will be pressurized with nitrogen. The following settings will be used for the GC/autosampler:

GC oven:	40 °C
GC cycle	3.5 minutes

Front inlet	110 °C	
Pressure	10 psi	
Total flow	55.948 mL/min	
Split ratio	10:1	
FID settings:		300 °C
	H_2 flow	30 mL/min
	Air flow	400 mL/min
	Make up flow (N_2)	25 mL/min
Vial oven	60 °C	
Loop	60 °C	
Transfer line	90 °C	
Vial equil.	22 minutes	
Vial shaking	off	
Vial fill	15 psi	
Loop ramp	30 psi/min	

Performance Characteristics

The instrument/method will be evaluated using the following performance characteristics. Additional testing outside of the testing described in this plan may be done as part of evaluating the instrument's performance. Any additional testing will be detailed when describing the outcome of the validation testing. The retention time of a peak must be within 0.04 minutes of the retention time observed for ethanol in the calibration standards for a peak to be identified as ethanol.

- **Calibration model**

The applicability of a linear calibration model will be tested using data from five runs on five different days. Aqueous ethanol solutions will be used to assess the calibration model. The following calibrator concentrations will be used in each run: 0.02 g/dL, 0.10 g/dL, 0.15 g/dL, 0.20 g/dL, 0.30 g/dL, and 0.40 g/dL. The data points from the five runs will be plotted together to establish the calibration model. The origin will not be included as a calibration point.

The calibration model will be evaluated by looking at both the correlation coefficient (r) and the residuals. The r^2 values must be at least 0.995.

Outside of establishing the calibration model, the linearity of the detector response will be tested over a range of concentration from 0.01 g/dL to 0.50 g/dL. Three runs on three different days will be conducted using the following calibrator concentrations: 0.01 g/dL, 0.10 g/dL, 0.20 g/dL, 0.30 g/dL, 0.40 g/dL, and 0.50 g/dL. The linearity of detector response will be evaluated based on the correlation coefficient (r). The r^2 value must be at least 0.995.

- **Bias**

Bias will be evaluated using at least 3 separate samples per concentration per run in at least three separate runs. At least the following approximate ethanol concentrations will be used in the analysis of bias; 0.04 g/dL, 0.08 g/dL, 0.15 g/dL, 0.2 g/dL, and 0.4 g/dL. Both aqueous and whole blood reference materials will be used to assess bias. A bias of less than or equal to ± 5% is acceptable.

- **Precision**

Both within-run and between-run precision will be evaluated. Precision will be evaluated using at least 3 separate samples per concentration per run in at least three separate runs. At least, the following approximate ethanol concentrations will be used in the analysis of precision; 0.04 g/dL, 0.08 g/dL, 0.15 g/dL, 0.2 g/dL, and 0.4 g/dL. Both aqueous and whole blood reference materials will be used to assess precision. A percent coefficient of variation less than or equal to ± 10% is acceptable.

- **Carryover**

The potential for carryover will be evaluated by running a blank sample following a sample containing 0.50 g/dL ethanol. This test will be evaluated using three runs on three different days.

- **Interference studies**

Evaluating matrix interference

Human blood drawn into a vacutainer tube will be analyzed to evaluate the possibility of matrix interference. The blood sample will be analyzed without the presence of the internal standard to demonstrate the absence of interferences from the matrix.

Evaluating interference from other volatile organic compounds

The possible interference of the following volatiles will be studied: acetaldehyde, methanol, acetone, and isopropanol. The instrument must exhibit baseline separation of these volatiles from ethanol and n-propanol. In addition, the possible effect that a sample containing toluene might have an analysis of subsequent samples will be evaluated by analyzing control samples following samples containing toluene.

- **Limit of detection/Limit of quantitation**

The limit of detection will be determined by using the administratively-defined decision point of 0.01 g/dL. At least three samples of 0.01 g/dL ethanol solution per run will be examined on three different days. All detection and identification criteria must be met.

The limit of quantitation will be set as the lowest non-zero calibrator (i.e. 0.02 g/dL). At least three samples of 0.02 g/dL ethanol solution per run will be examined on three different days. All detection, identification, bias, and precision criteria must be met.

Documentation

The data generated during the validation study will be maintained and made available for review. The results of the validation study will be summarized and presented for review.

APPENDIX TWO

Cerilliant
Analytical Reference Standards
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ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-10

Ethyl Alcohol

Catalog Number:	E-040
Solution Lot:	FN080612-04
Expiration Date:	August 2017
Diluent:	Water
Volume per Ampoule:	1.2 mL
Storage:	Refrigerate. Do not freeze.
Intended Use:	For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	10.00 ± 0.04 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

REFERENCE MATERIAL PRODUCER

Lara Sparks

Lara Sparks, Quality Assurance Director

October 11, 2012
Date

Cerilliant®
Analytical Reference Standards
a SIGMA-ALDRICH company

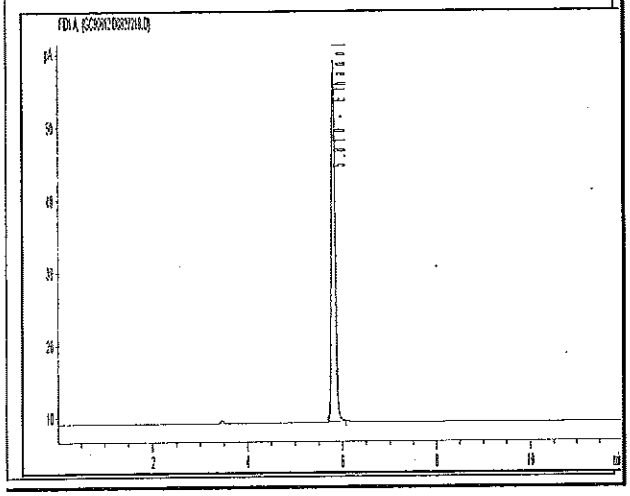
Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2891 (mg/dL)	Results compared to Control (% Difference)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN080612-04	9.86	1.43%	0.53%
Acceptance Criteria		±2%	±2%	≤2%
Prior Lot	FN111110-01	9.712	Relative Standard Uncertainty of Method: 1.675%	

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

Solution Standard Assay Parameters

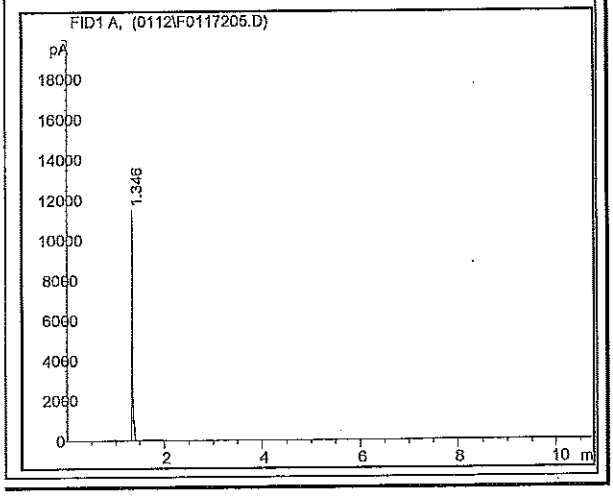
Analysis Method:	GC/FID Headspace
Column:	DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness
Temp Program:	40°C hold for 12 min
Injector Temp:	200°C
Detector Temp:	250°C



Neat Material Analysis

Purity by GC/FID Analysis:	100.0%
Water Content by Karl Fischer:	0.10%
Purity Factor:	99.90%

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



Cerilliant®

Cerilliant Quality
ISO GUIDE 34
ACCREDITED
CERTIFICATE AR1353
ISO/IEC 17025
ACCREDITED
CERTIFICATE AR1352
ISO 9001:2000
CERTIFIED
CERTIFICATE 3854

Certificate of Analysis

Certified Reference Material - NIST Traceable

Ethanol-20

Ethyl Alcohol

Catalog Number:	E-056
Solution Lot:	FN030409-01
Expiration Date:	March 2014
Diluent:	Water
Volume per Ampule:	1.2 mL
Storage:	Protect from light, refrigerate. Do not freeze.
Intended Use:	For laboratory use only. Not suitable for human or animal consumption.

- Expiration Date has been established through real time stability studies.
- Ampules are overfilled to ensure a minimum 1.2 mL volume fill. We advise laboratories to use measured volumes of this solution standard before diluting to the desired concentration.

Component	Chromatographic Purity	Concentration
Ethanol	100%	20.00 ± 0.07 mg/dL

- Chromatographic purity of the solution is verified post ampuling to provide assurance of no contamination or degradation during manufacturing.
- Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO/IEC 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production system. Uncertainty includes uncertainty of the purity factor, material density and mass. Purity factor uncertainty incorporates uncertainty of all analyses performed to characterize the raw material including chromatographic purity and residual water. Mass uncertainty incorporates uncertainty of the balance in its installed environment and weighing technique and was determined through repeatability experiments using Cerilliant established weighing procedures.
- This standard meets the definition of a Certified Reference Material in accordance with ISO Guide 34.

Traceability

- This standard and its preparation are fully traceable to the SI through NIST.
- This standard was gravimetrically prepared using qualified balances calibrated semi-annually by Mettler Toledo, an ISO/IEC 17025 accredited company, using NIST traceable weights. Calibration verification is performed weekly through the range of the balance and then prior to each use. All calibration verifications are performed utilizing NIST traceable weights which are externally calibrated on an annual basis by a qualified ISO 17025 accredited calibration laboratory. Weigh tapes verifying pre-use balance calibration are included in the production batch record for this standard. Each balance has been assigned a minimum weighing by Mettler Toledo taking into consideration the balance and installed environmental conditions to ensure weighing complies with USP tolerances of no more than 0.1% relative error.
- Concentration is analytically verified by multiple analyses directly to a NIST SRM.

Cerilliant certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration date when stored unopened as recommended. Product should be used shortly after opening to avoid concentration changes due to evaporation. Warranty does not apply to ampoules stored after opening.

ACLASS

Lara Sparks

Lara Sparks, Quality Assurance Director

April 27, 2009
Date

Analytical Verification of Solution Standard Concentration and Homogeneity

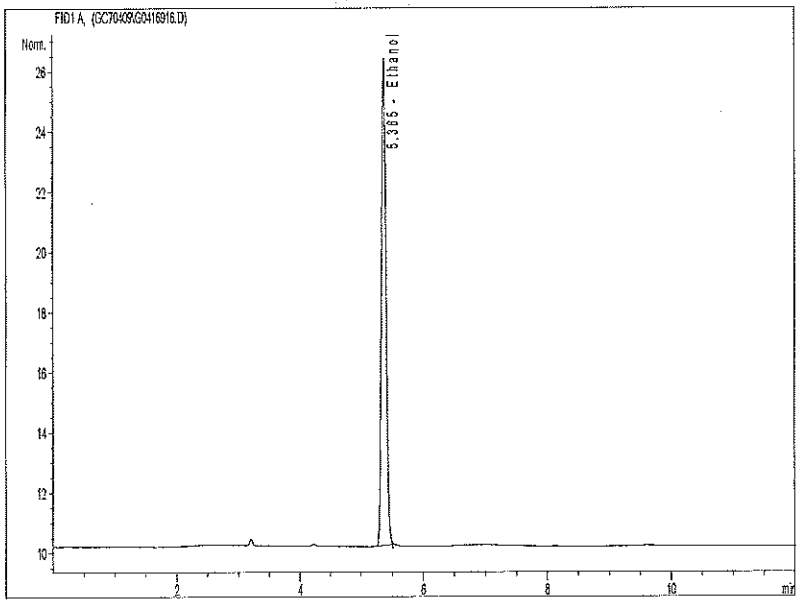
Solution Standard	Lot Number	Concentration (mg/dL)	NIST SRM Lot and Concentration used for Assay	%RSD	
New Lot	FN030409-01	20.41	SRM 2891	1.1%	Homogeneity
Prior Lot	FN022207-02	20.02	0.01951% ± 0.00018%	2.3%	System Suitability

- Concentration is calculated as the average of multiple analyses by GC Headspace compared directly to the NIST SRM lot listed above. Acceptance criteria of ±2.0% incorporates variability of the analysis. Concentration of the NIST SRM lot is as certified by NIST.
- Homogeneity of the New Lot is ensured through the use of validated processes and verified by analysis. The %RSD of samples pulled from across the lot demonstrates homogeneity of the New Lot.
- The %RSD of the Prior Lot represents variability of the analysis performed during solution standard release testing and system suitability. Triplicate injections of the Prior Lot are bracketed at the beginning and end of the sequence. %RSD criteria of ≤2% ensures system performance throughout the sequence.
- All testing equipment is fully qualified through an installation qualification and annual operational qualifications.

Solution Standard Assay Parameters

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

Raw Material Verification by GC/FID



COA Revision History

Revision No.	Date	Reason for Revision
00	04/27/2009	Initial version.
01	03/04/2010	Corrected Concentration on page 2 from mg/mL to mg/dL.

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Analytical Reference Standards
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ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-20

Ethyl Alcohol

Catalog Number:	E-056
Solution Lot:	FN09031301
Expiration Date:	September 2018
Diluent:	Water
Volume per Ampoule:	1.2 mL
Storage:	Refrigerate. Do not freeze.
Intended Use:	For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	20.00 ± 0.07 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

A2LA ACCREDITED
REFERENCE MATERIAL PRODUCER

Darron Ellsworth, Quality Assurance Manager

February 13, 2009
Date

Cerilliant®
Analytical Reference Standards
a SIGMA-ALDRICH company

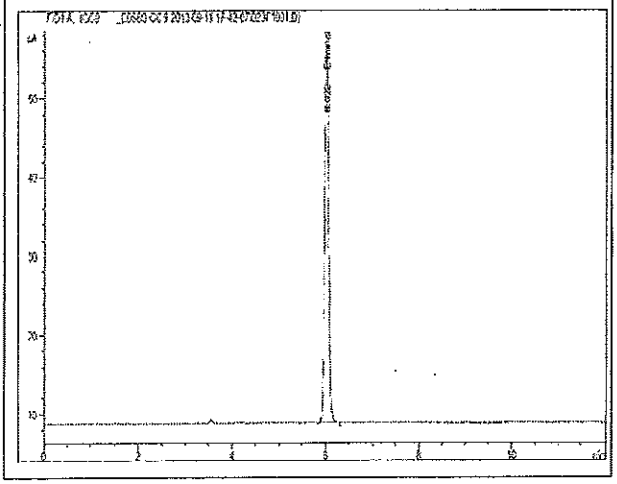
Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2891 (mg/dL)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN09031301	20.02	0.66%
Prior Lot	FN092710-01	19.89	0.90%
Acceptance Criteria		±2%	≤2%

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

Solution Standard Assay Parameters

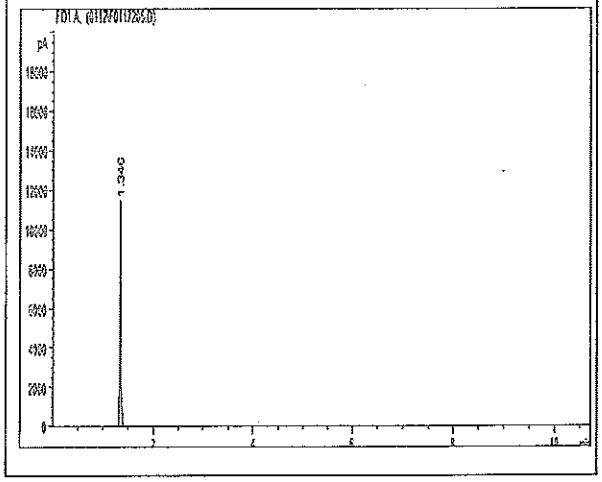
Analysis Method:	GC/FID Headspace
Column:	DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness
Temp Program:	40°C hold for 12 min
Injector Temp:	200°C
Detector Temp:	250°C



Neat Material Analysis

Purity by GC/FID Analysis:	100.0%
Water Content by Karl Fischer:	0.1%
Purity Factor:	99.9%

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



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Analytical Reference Standards
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ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-100

Ethyl Alcohol

Catalog Number: E-031
Solution Lot: FN050312-01
Expiration Date: May 2017
Diluent: Water
Volume per Ampoule: 1.2 mL
Storage: Refrigerate. Do not freeze.
Intended Use: For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	100.0 ± 0.4 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

REFERENCE MATERIAL PRODUCER

Lara Sparks

Lara Sparks, Quality Assurance Director

May 31, 2012
Date

Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2894 (mg/dL)	Results compared to Control (% Difference)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN050312-01	98.49	0.78%	0.75%
Prior Lot	FN111711-01	98.70	0.58%	1.45%
Acceptance Criteria		±2%	±2%	≤2%

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

Solution Standard Assay Parameters

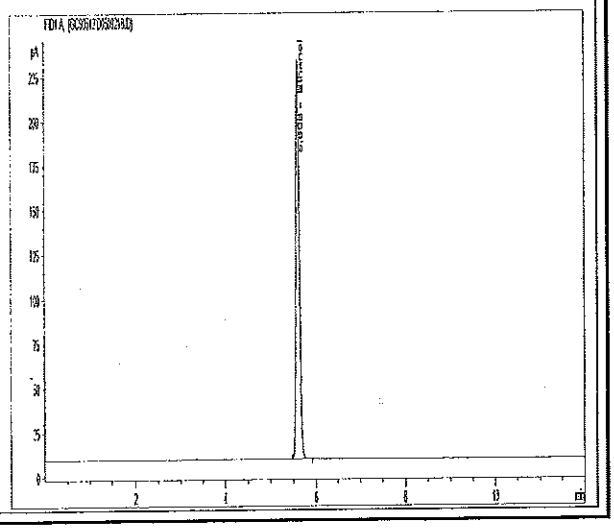
Analysis Method: GC/FID Headspace

Column: DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness

Temp Program: 40°C hold for 12 min

Injector Temp: 200°C

Detector Temp: 250°C



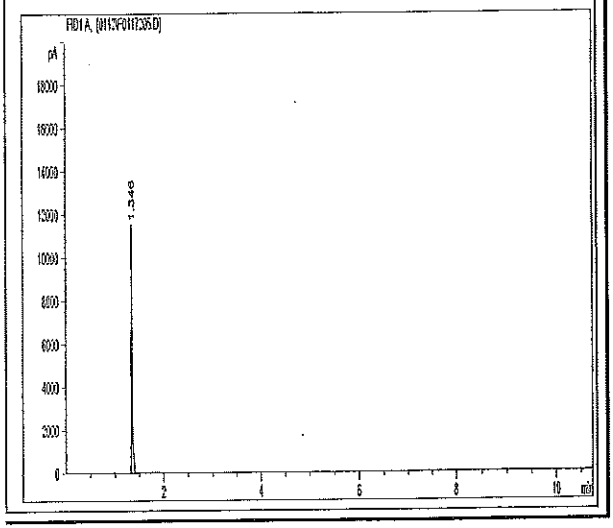
Neat Material Analysis

Purity by GC/FID Analysis: 100.0%

Water Content by Karl Fischer: 0.10%

Purity Factor: 99.90%

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



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ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-150

Ethyl Alcohol

Catalog Number: E-041
Solution Lot: FN102912-02
Expiration Date: October 2017
Diluent: Water
Volume per Ampoule: 1.2 mL
Storage: Refrigerate. Do not freeze.
Intended Use: For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	150.0 ± 0.5 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

ACLASS ACCREDITED
REFERENCE MATERIAL PRODUCER

Lara Sparks

Lara Sparks, Quality Assurance Director

November 29, 2012
Date

Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2895 (mg/dL)	Results compared to Control (% Difference)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN102912-02	148.9	0.99%	0.73%
Prior Lot	FN091310-04	147.5	1.90%	0.21%
Acceptance Criteria		±2%	±2%	≤2%

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

Solution Standard Assay Parameters

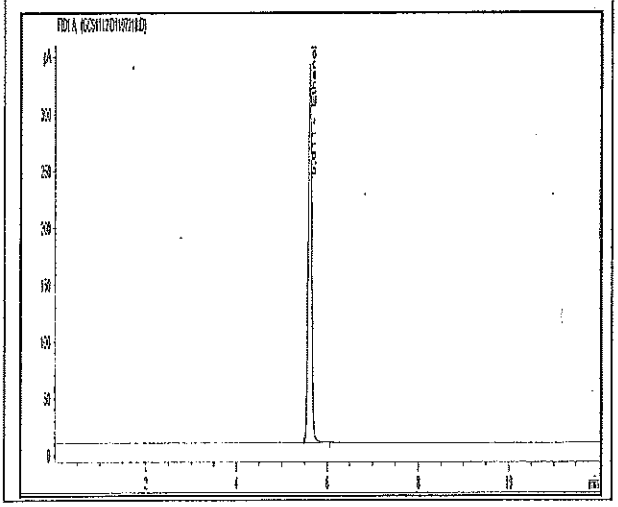
Analysis Method: GC/FID Headspace

Column: DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness

Temp Program: 40°C hold for 12 min

Injector Temp: 200°C

Detector Temp: 250°C



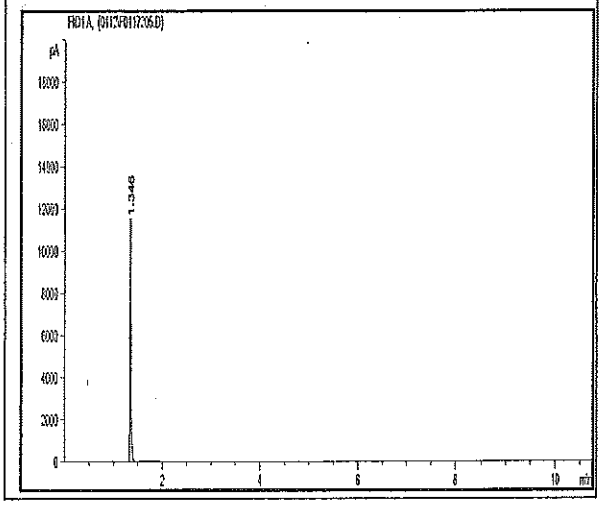
Neat Material Analysis

Purity by GC/FID Analysis: 100.0%

Water Content by Karl Fischer: 0.10%

Purity Factor: 99.90%

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



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Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-200

Ethyl Alcohol

Catalog Number: E-032
Solution Lot: FN032712-01
Expiration Date: March 2017
Diluent: Water
Volume per Ampoule: 1.2 mL
Storage: Refrigerate. Do not freeze.
Intended Use: For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	200.0 ± 0.7 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

ACLASS ACCREDITED
REFERENCE MATERIAL PRODUCER

Lara Sparks, Quality Assurance Director

May 6, 2012
Date

Cerilliant
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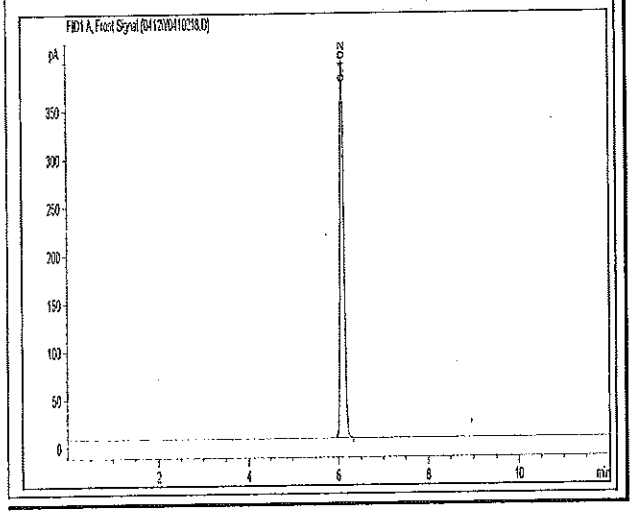
Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2895 (mg/dL)	Results compared to Control (% Difference)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN032712-01	200.3	0.14%	0.70%
Prior Lot	FN070209-01	199.0	0.76%	0.50%
Acceptance Criteria		±2%	±2%	≤2%

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

Solution Standard Assay Parameters

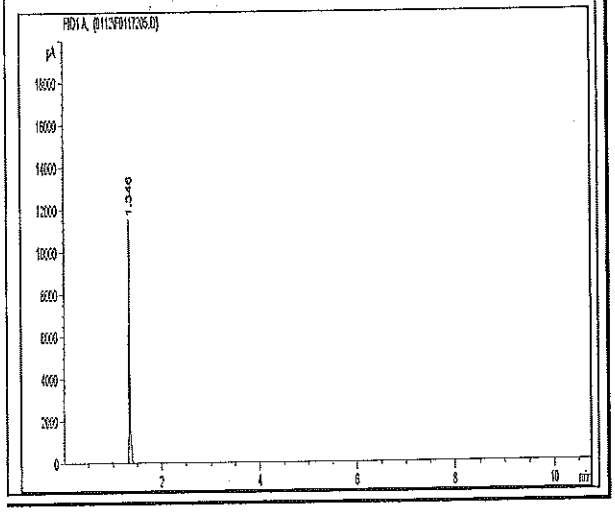
Analysis Method:	GC/FID Headspace
Column:	DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness
Temp Program:	40°C hold for 12 min
Injector Temp:	200°C
Detector Temp:	250°C



Neat Material Analysis

Purity by GC/FID Analysis:	100.0%
Water Content by Karl Fischer:	0.10%
Purity Factor:	99.90%

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



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Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-300

Ethyl Alcohol

Catalog Number:	E-033
Solution Lot:	FN09061305
Expiration Date:	October 2018
Diluent:	Water
Volume per Ampoule:	1.2 mL
Storage:	Refrigerate. Do not freeze.
Intended Use:	For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	300.0 ± 1.06 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 17025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

REFERENCE MATERIAL PRODUCER

Darron Ellsworth, Quality Assurance Manager

January 10, 2014
Date

Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2896 (mg/dL)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN09061305	301.13	0.79%
Prior Lot	FN121510-01	301.64	0.73%
Acceptance Criteria		±2%	≤2%

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

Solution Standard Assay Parameters

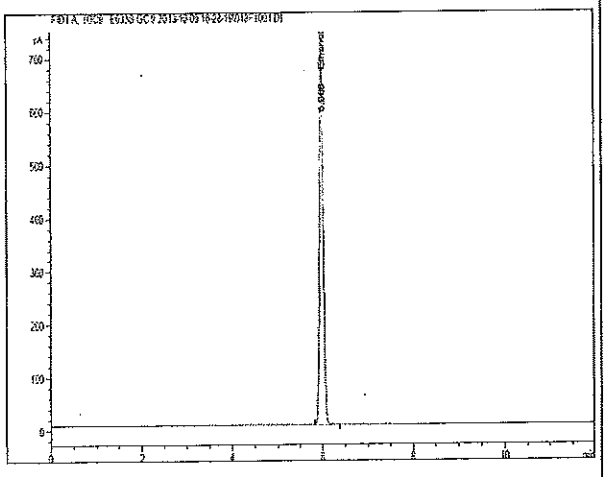
Analysis Method: GC/FID Headspace

Column: DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness

Temp Program: 40°C hold for 12 min

Injector Temp: 200°C

Detector Temp: 250°C



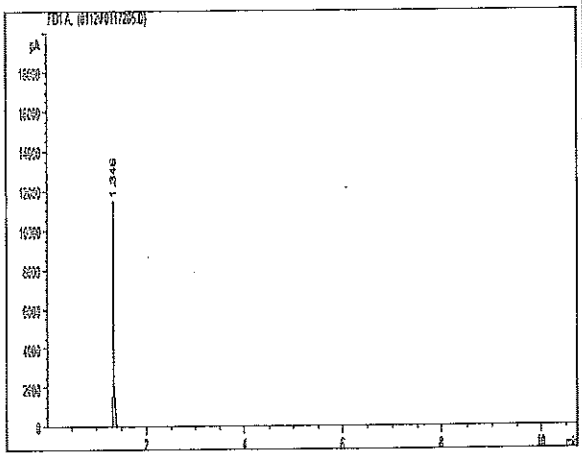
Neat Material Analysis

Purity by GC/FID Analysis: 100.0%

Water Content by Karl Fischer: 0.1%

Purity Factor: 99.9%

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



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Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-400

Ethyl Alcohol

Catalog Number:	E-036
Solution Lot:	FN012712-01
Expiration Date:	January 2017
Diluent:	Water
Volume per Ampoule:	1.2 mL
Storage:	Refrigerate. Do not freeze.
Intended Use:	For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	400.0 ± 1.4 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

ACCREDITED
REFERENCE MATERIAL PRODUCER

Lara Sparks

Lara Sparks, Quality Assurance Director

February 20, 2012
Date

Cerilliant
Analytical Reference Standards
a SIGMA-ALDRICH Company

Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2896 (mg/dL)	Results compared to Control (% Difference)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN012712-01	399.9	0.19%	0.72%
Prior Lot	FN040909-01	397.6	0.39%	1.09%
Acceptance Criteria		±2%	±2%	≤2%

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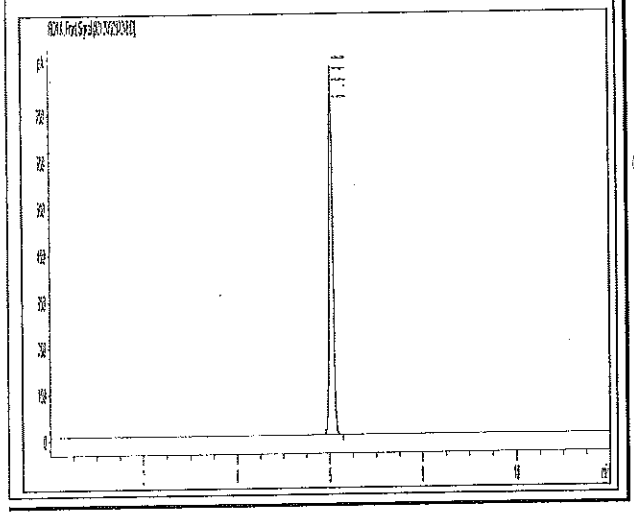
- The %RSD of the Prior Lot represents system suitability on the date of analysis. Triplicate injections of the Prior Lot are bracketed at the beginning and end of the sequence. %RSD criteria ensures proper system performance throughout the sequence.
- All instruments used for certification of the neat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

Solution Standard Assay Parameters

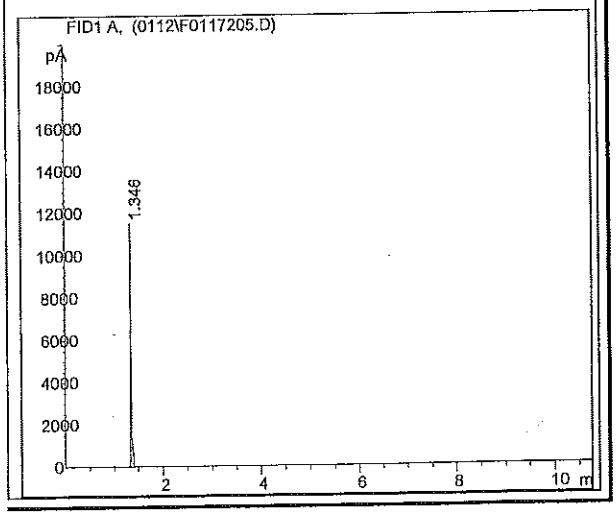
Analysis Method:	GC/FID Headspace
Column:	DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness
Temp Program:	40°C hold for 12 min
Injector Temp:	200°C
Detector Temp:	250°C



Neat Material Analysis

Purity by GC/FID Analysis:	100.0%
Water Content by Karl Fischer:	0.10%
Purity Factor:	99.90%

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



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Certificate of Analysis

Certified Reference Standard - NIST Traceable

Ethanol-500

Ethyl Alcohol

Catalog Number:	E-053
Solution Lot:	FN012813-01
Expiration Date:	January 2018
Diluent:	Water
Volume per Ampoule:	1.2 mL
Storage:	Refrigerate. Do not freeze.
Intended Use:	For R&D/ analytical purposes only. Not suitable for human or animal consumption.

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

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	100%	500.0 ± 1.8 mg/dL

- Uncertainty of the concentration, expressed in terms of volume, is an expanded uncertainty in accordance with ISO 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production methods applicable to ethanol reference standards and incorporates uncertainty of the purity factor, material density and mass measurement. The dispensing process is sufficiently controlled as to not be a significant contributor to uncertainty calculations and is, therefore, excluded. Solution stability is established through real time stability studies and is, therefore, excluded.
- When expressed in percentage terms, the relative standard uncertainty of the concentration is 0.175% and the relative expanded uncertainty is 0.35% at the 95% confidence interval (k=2).
- The purity factor (PF) mass balance measurement equation is used to calculate the amount of ethanol required to achieve an accurate concentration of the solution standard, accounting for both purity and residual water content.
- Purity factor has been established through independent certification of the neat analyte to ISO 172025 standards – See page 2.
- Solution purity is verified post ampouling and demonstrates no contamination or degradation has occurred.

Traceability to SI through NIST:

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

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

REFERENCE MATERIAL PRODUCER

Lara Sparks

Lara Sparks, Quality Assurance Director

March 7, 2013

Date

Cerilliant
Analytical Reference Standards
a SIGMA-ALDRICH company

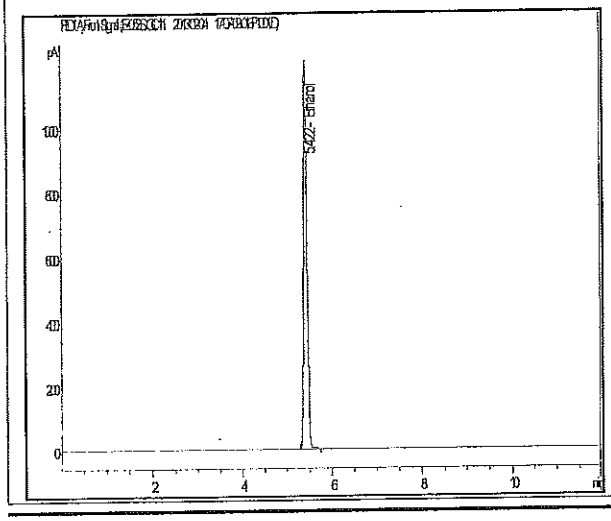
Analytical Verification of Solution Standard Concentration and Batch Homogeneity:

Solution Standard	Lot Number	Results compared to NIST SRM Lot 2896 (mg/dL)	Results compared to Control (% Difference)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	FN012813-01	498.0	0.39%	0.84%
Prior Lot	FN102710-01	496.1	0.78%	1.13%
Acceptance Criteria		±2%	±2%	≤2%

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

Solution Standard Assay Parameters

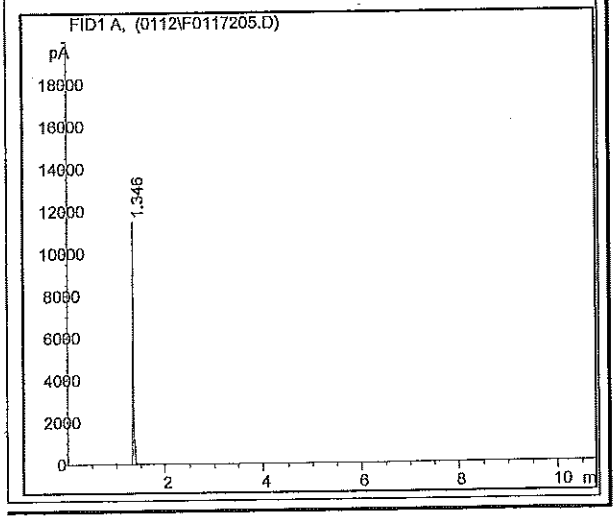
Analysis Method:	GC/FID Headspace
Column:	DB-ALC1 30 m x 0.53 mm ID, 3.0 µm film thickness
Temp Program:	40°C hold for 12 min
Injector Temp:	200°C
Detector Temp:	250°C



Neat Material Analysis

Purity by GC/FID Analysis:	100.0%
Water Content by Karl Fischer:	0.10%
Purity Factor:	99.90%

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



Cerilliant®

ISO GUIDE 34 CERTIFICATE AR1355
ISO/IEC 17025 CERTIFICATE AT1352
ISO 9001:2008 CERTIFICATE 3854

Certificate of Analysis

Multicomponent Alcohol Calibration Kit – NIST Traceable

Catalog Number: A-054
Solution Lot: FN122210-01
Expiration Date: December 2015
Diluent: Water
Volume per Ampule: 1.2 mL
Storage: Refrigerate. Do not freeze.
Intended Use: For laboratory use only. Not suitable for human or animal consumption.

- Expiration Date has been established through real time stability studies.
- Ampules are overfilled to ensure a minimum 1.2 mL volume fill. We advise laboratories to use measured volumes of this solution standard before diluting to the desired concentration.

Component	Purity	Concentration		
		C1 Level	C2 Level	C3 Level
Acetone	99%	500.0 ± 1.8 µg/mL	1000 ± 3.6 µg/mL	4000 ± 14.4 µg/mL
Methanol	99%	500.0 ± 1.8 µg/mL	1000 ± 3.6 µg/mL	4000 ±14.4 µg/mL
Ethanol	99%	500.0 ± 1.8 µg/mL	1000 ± 3.6 µg/mL	4000 ±14.4 µg/mL
Isopropanol	99%	500.0 ± 1.8 µg/mL	1000 ± 3.6 µg/mL	4000 ±14.4 µg/mL

- Chromatographic purity of each component in the standard solution is verified post ampuling to provide assurance of no contamination or degradation during manufacturing.
- Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO/IEC 17025 and ISO Guide 34 at the 95% confidence interval using a coverage factor of k=2 and has been calculated by statistical analysis of our production system. Uncertainty includes uncertainty of the purity factor, material density and mass (including balance, environmental conditions, and weighing technique).

Traceability

- This standard and its preparation are fully traceable to the SI through NIST.
- This standard was gravimetrically prepared using qualified balances calibrated semi-annually by Mettler Toledo, an ISO/IEC 17025 accredited company, using NIST traceable weights. Calibration verification is performed weekly through the range of the balance and then prior to each use. All calibration verifications are performed utilizing NIST traceable weights which are externally calibrated on an annual basis by a qualified ISO 17025 accredited calibration laboratory. Weigh tapes verifying pre-use balance calibration are included in the production batch record for this standard. Each balance has been assigned a minimum weighing by Mettler Toledo taking into consideration the balance and installed environmental conditions to ensure weighing complies with USP tolerances of no more than 0.1% relative error.
- Concentration of each component is analytically verified by multiple analyses to an independently prepared multi-point calibration curve. The ethanol calibration points are prepared from a NIST SRM.

Cerilliant certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration date when stored unopened as recommended. Product should be used shortly after opening to avoid concentration changes due to evaporation. Warranty does not apply to ampoules stored after opening.

Lara Sparks, Quality Assurance Director

February 23, 2011
Date

Analytical Verification of Solution Standard Concentration and Homogeneity

Acetone

	C1 Level			C2 Level			C3 Level		
	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD
New Lot	FN122210-01A	506.3	0.7	FN122210-01B	987	0.3	FN122210-01C	3996	0.6
Prior Lot	FN030909-01A	501.9	0.5	FN030909-01B	981	0.3	FN030909-01C	3983	0.6

Methanol

	C1 Level			C2 Level			C3 Level		
	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD
New Lot	FN122210-01A	498.8	1.0	FN122210-01B	990	2.0	FN122210-01C	4003	0.9
Prior Lot	FN030909-01A	497.0	1.6	FN030909-01B	974	1.3	FN030909-01C	4183	2.3

Isopropanol

	C1 Level			C2 Level			C3 Level		
	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD
New Lot	FN122210-01A	500.8	0.6	FN122210-01B	986	0.6	FN122210-01C	3990	0.6
Prior Lot	FN030909-01A	500.1	0.5	FN030909-01B	980	0.5	FN030909-01C	4001	0.8

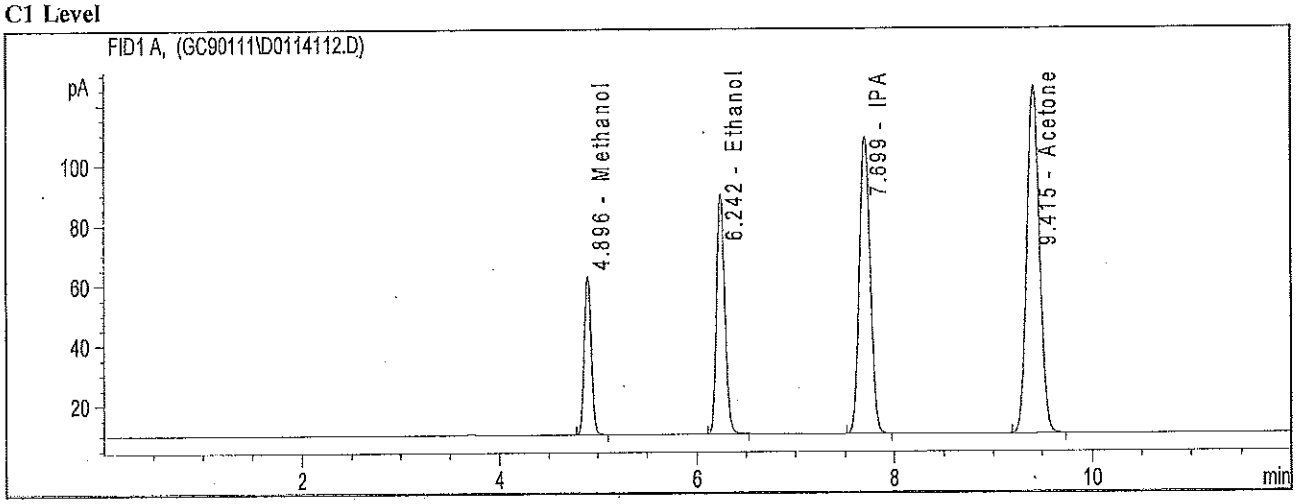
Ethanol

	C1 Level			C2 Level			C3 Level		
	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD	Lot Number	Concentration (µg/mL)	%RSD
New Lot	FN122210-01A	497.2	1.1	FN122210-01B	975	0.8	FN122210-01C	3957	0.8
Prior Lot	FN030909-01A	498.7	0.2	FN030909-01B	991	1.1	FN030909-01C	3999	1.0

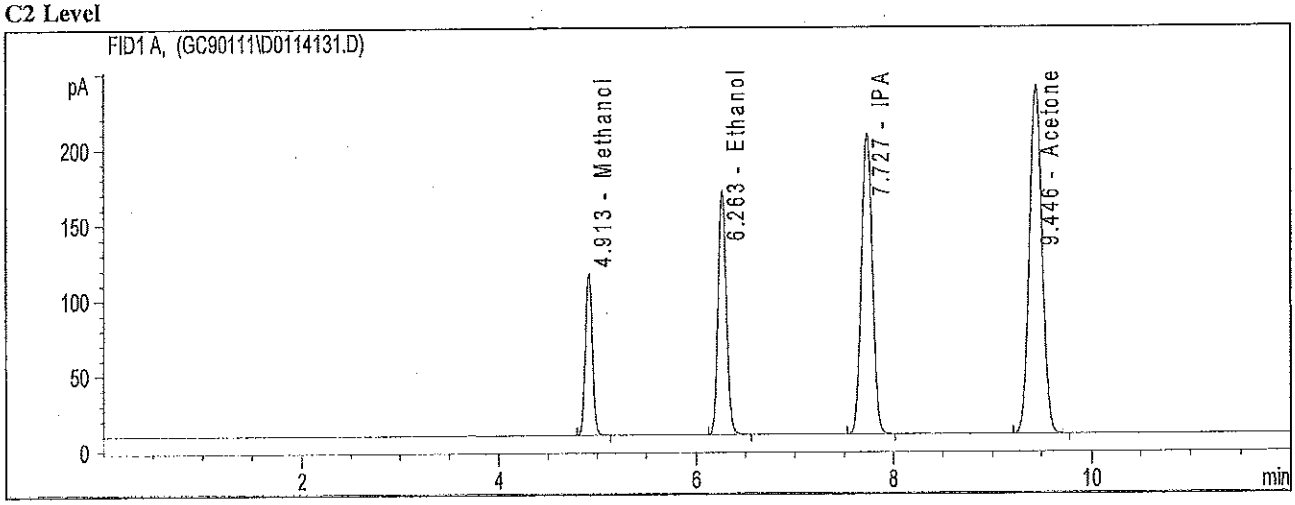
- Concentration is calculated as the average of multiple analyses by GC Headspace compared to an independently prepared calibration solution. The ethanol calibration points were prepared from NIST SRM 2894 Acceptance criteria of ±5.0% incorporates variability of the analysis.
- Homogeneity of the New Lot is ensured through the use of validated processes and verified by analysis. The %RSD of samples pulled from across the lot demonstrates homogeneity of the New Lot.
- The %RSD of the Prior Lot represents variability of the analysis performed during solution standard release testing and system suitability. Triplicate injections of the Prior Lot are bracketed at the beginning and end of the sequence. %RSD criteria of ≤2% ensures system performance throughout the sequence.
- All testing equipment is fully qualified through an installation qualification and annual operational qualifications.

Analytical Verification

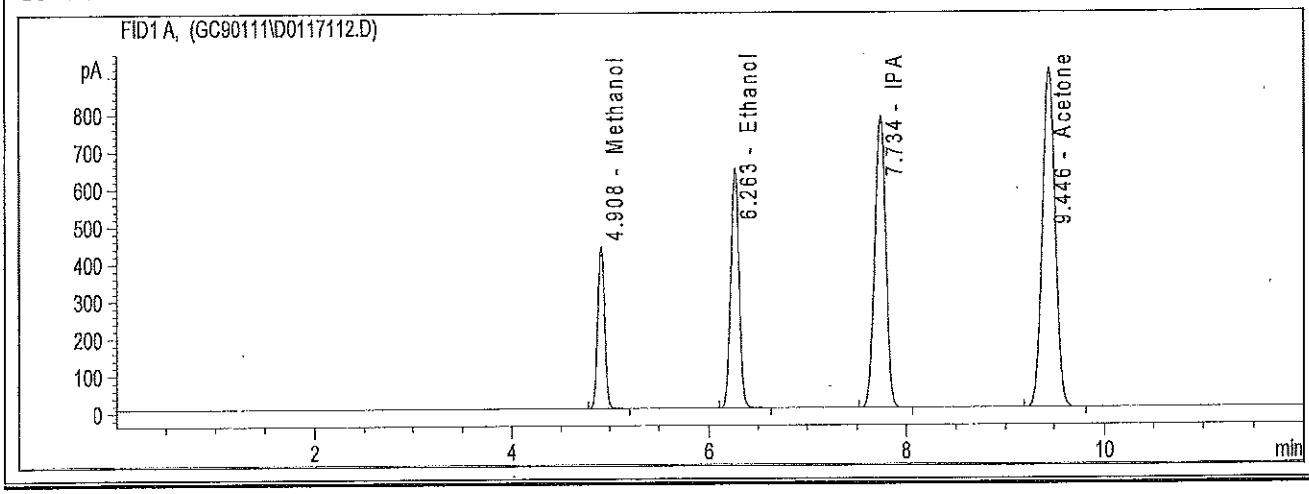
C1 Level



C2 Level



C3 Level



Certificate of Analysis

SIGMA-ALDRICH

Product Name	Toluene, ACS reagent, ≥99.5%
Product Number	179418
Product Brand	SIAL
CAS Number	108-88-3
Molecular Formula	$C_6H_5CH_3$
Molecular Weight	92.14

TEST	SPECIFICATION	LOT 02302PW RESULTS
APPEARANCE	COLORLESS LIQUID	COLORLESS LIQUID
REFRACTIVE INDEX AT 20 DEG C		1.4956
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.	CONFORMS TO STRUCTURE AND STANDARD AS ILLUS- TRATED ON PAGE 931B OF EDITION I, VOLUME 1 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".
GAS LIQUID CHROMATOGRAPHY	99.50% (MINIMUM)	99.9 %
TITRATION	0.03% H2O (MAXIMUM)	0.02 % H2O
RESIDUE ON EVAPORATION	0.001% (MAXIMUM)	0.0008 %
COLOR TEST	10 APHA (MAXIMUM)	APHA<10
FREE HALOGENS		PASSES TEST
SULFUR COMPOUNDS	0.003% (MAXIMUM) AS S	<0.003 %

Barbara Rajzer

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Whole Blood Ethanol Control Level 1

CE

INTENDED USE
FOR IN VITRO DIAGNOSTIC USE

LiquiSP$_x$™ Whole Blood Ethanol Control is an assayed quality control material intended for use in monitoring the accuracy and precision of the quantitative determination of ethanol in whole blood.

SUMMARY AND PRINCIPLE
This product is to be used exactly as directed for the patient sample in order to monitor, and thus minimize the potential for technical and performance errors in routine testing.

PRODUCT DESCRIPTION
LiquiSP$_x$ Whole Blood Ethanol Control is prepared from stabilized normal human whole blood with the addition of ethanol. This product has been assigned lot-specific ethanol values using quantitative analytical methods. This product is packaged 5.0 mL per vial.

2°C – 8°C

STORAGE AND STABILITY
LiquiSP$_x$ Whole Blood Ethanol Control is stable until the expiration date on the package when stored unopened at 2-8° C and 45 days after opening when stored at 2-8° C. Discard any contaminated material. Microbial contamination is evidenced by an increase in turbidity and/or a characteristic odor.

⚠ PRECAUTIONS
Human source material. Treat as potentially infectious.

Each serum/plasma donor unit used in the manufacture of this product has been tested by FDA accepted methods and found non-reactive for the presence of HBsAg and antibody to HIV-1/2, HCV and HIV-1 Ag. While these methods are highly accurate, they do not guarantee that all infected units will be detected. Because no known test method can offer complete assurance the hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV) or other infectious agents are absent, all products containing human source material should be considered potentially infectious and handled with the same precautions used with patient specimens.

This product contains 0.09% sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form potentially explosive compounds. Flush with excess water upon disposal.

PROCEDURE
Allow the refrigerated controls to warm to room temperature (18-25° C) and gently swirl the control material prior to use in order to ensure product homogeneity. QC materials should be used in accordance with local, state, and/or federal regulations or accreditation requirements.

LIMITATIONS
This material is a control for methods listed in the ASSIGNED VALUES section; it is not to be used as a calibrator. Accurate and reproducible results are dependent upon properly functioning instruments, reagents, standardization, and proper laboratory techniques. Individual laboratories may not obtain the mean assigned value as listed.

VALUE ASSIGNMENT
The mean values and expected ranges printed in this insert were derived from extensive replicate analyses and are specific for this lot.

Values listed below were generated by Cliniqa, the reagent/instrument manufacturer and/or independent laboratories in accordance with an established protocol.

Procedural or assay modifications may alter the mean value obtained. Each laboratory should establish its own parameters of precision; use the mean assigned values and expected ranges provided only as guidelines.

ASSIGNED VALUES

Level 1		Lot No.: 1212090 Exp. Date: 2016-12	
Method	**Units**	**Mean**	**Expected Range**
Gas Chromatography	mg/dL	77.3	61.8 - 92.7

REFERENCES
Baselt, R.C. Analytical Procedures for Therapeutic Drug Monitoring and Emergency Toxicology. Littleton, MA, PSG Publishing, 1987.

IVD For in vitro diagnostic use

See package insert for proper use

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RE-ORDER INFORMATION
Whole Blood Ethanol Control

Catalog No.	Catalog No.	Catalog No.
REF 93211	REF 93212	REF 93213
Level 1, 6 x 5 mL	Level 2, 6 x 5 mL	Level 3, 6 x 5 mL

CLINIQA

EtOH WH 2,0 g/l – Uso diagnostico in vitro

Controllo d'etanolo in sangue intero

Applicazione
Utilizzabile nelle procedure definite da ciascun laboratorio come calibratore o come materiale di controllo.

Utilizzo
Pronto all'uso.

Valori attesi
I valori assegnati sono stati determinati da 3 laboratori indipendenti accreditati (DIN EN 17025) attraverso la misurazione in duplicato tramite il metodo cromatografico GC per 5 giorni.

Conservazione e stabilità
Conservazione: + 2° fino a + 8° C
Stabilità:
- Flacone non aperto: se conservato ben chiuso ed al riparo dalla luce fino alla data di scadenza.
- Flacone aperto: se conservato ben chiuso ed al riparo dalla luce fino alla data di scadenza in etichetta.

Precauzioni
I componenti originari da cui questo prodotto è stato derivato, sono stati trovati negativi per HBsAg e per gli anticorpi contro HCV, HBc, HIV-1 e HIV-2 attraverso metodologie di analisi approvate.
Tuttavia, poiché nessuna analisi può offrire sicurezza completa che gli agenti infettivi siano assenti, questo prodotto deve essere manipolato osservando le stesse precauzioni di sicurezza usate quando si manipola qualunque tipo di materiale potenzialmente infettivo.

Lotto: 402101060
Codice: WH20-015 (10 x 1,5 ml)
WH20-030 (10 x 3,0 ml)
Versione: 3 – 201303

EtOH WH 2,0 g/L – Usage in vitro

Contrôle d'éthanol dans le sang total

Application
Standard dédié à la calibration pour techniques analytiques de détermination de concentration d'éthanol ou a utiliser comme contrôle d'exactitude.

Utilisation
Ce contrôle est prêt à l'emploi.

Valeur cible
Les valeurs cibles ont été déterminées par 3 laboratoires accrédités (DIN EN 17025). Une double détermination a été effectuée par jour par méthode chromatographique GC pendant 5 jours.

Conservation et stabilité
Conservation: + 2° jusqu'à 8° C
Stabilité:
- Scellé (à l'origine), à l'abri de la lumière: voire la date d'expiration indiquée sur l'étiquette.
- à stocker hermétiquement à l'abri de la lumière: voire la date d'expiration indiquée sur l'étiquette.

Précautions
Tout matériel humain doit être considéré comme étant potentiellement infectieux et traité dans les mêmes conditions que des échantillons de patients.
Chaque unité de sang utilisée pour la préparation de ce contrôle a été testée et trouvée négative pour les antigènes et anticorps suivants: AgHBs, anti-HIV-1, anti-HIV-2, anti-HBc et anti-HCV.

Lot: 402101060
Référence: WH20-015 (10 x 1,5 ml)
WH20-030 (10 x 3,0 ml)
Version: 3 - 201303

Metodo Méthode	Valori attesi Valeur cible	Intervallo di fiducia / Intervalle de confiance			Unità Unité
		statistico / statistique[1]	forense / medicine légale [2]	clinico / clinique[3]	
GC	2,002	1,949 – 2,055	1,902 – 2,102	1,822 – 2,182	g/L

[1] **Intervallo di fiducia** - Valori di analisi
L'intervallo di fiducia indica l'intervallo entro il quale si trova il valore atteso con un livello di significatività del 95%.

[2] **Intervallo di fiducia** – Direttiva Forense Tedesca
[EtOH] ≥ 1,0 g/l → ± 5% del valore atteso
[EtOH] < 1,0 g/l → ± 0,05 g/l del valore atteso

Bibliografia:
Bundesgesundheitsamt (1986) - Richtlinie für die Blutalkoholbestimmung für forensische Zwecke.
DACH(23.04.2008) - Spezieller Leitfaden für die Blutalkoholbestimmung für forensische Zwecke – VA 0900-54 Version1

[3] **Intervallo di fiducia** – Direttiva dell' Ordine Nazionale Tedesca dei Medici
0,2 < [EtOH] ≤ 0,6 g/l → ± 15 % del valore atteso
0,6 < [EtOH] ≤ 5,0 g/l → ± 9 % del valore atteso

Bibliografia:
Richtlinien der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (15.02.2008)

[1] **Intervalle de confiance** – Valeurs des analyses
La marge de confiance est la marge dans laquelle la valeur cible se trouve avec une probabilité de 95%.

[2] **Intervalle de confiance** – Directives allemandes de la Médecine Légale
[EtOH] ≥ 1,0 g/L → ± 5% de la valeur cible
[EtOH] < 1,0 g/L → ± 0,05 g/L de la valeur cible

Littérature:
Bundesgesundheitsamt (1986) - Richtlinie für die Blutalkoholbestimmung für forensische Zwecke.
DACH(23.04.2008) - Spezieller Leitfaden für die Blutalkoholbestimmung für forensische Zwecke – VA 0900-54 Version1

[3] **Intervalle de confiance** – Directives allemandes cliniques
0,2 < [EtOH] ≤ 0,6 g/L → ± 15 % de la valeur cible
0,6 < [EtOH] ≤ 5,0 g/L → ± 9 % de la valeur cible

Littérature:
Richtlinien der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (15.02.2008)

Hersteller / Manufacturer
ACQ Science GmbH
Etzwiesenstraße 37
Tel.: + 49 (0) 7457 94 69 3 0

CE

Whole Blood Ethanol Control Level 3

CE

INTENDED USE

FOR IN VITRO DIAGNOSTIC USE

LiquiSP$_x$™ Whole Blood Ethanol Control is an assayed quality control material intended for use in monitoring the accuracy and precision of the quantitative determination of ethanol in whole blood.

SUMMARY AND PRINCIPLE

This product is to be used exactly as directed for the patient sample in order to monitor, and thus minimize the potential for technical and performance errors in routine testing.

PRODUCT DESCRIPTION

LiquiSP$_x$ Whole Blood Ethanol Control is prepared from stabilized normal human whole blood with the addition of ethanol. This product has been assigned lot-specific ethanol values using quantitative analytical methods. This product is packaged 5.0 mL per vial.

STORAGE AND STABILITY

LiquiSP$_x$ Whole Blood Ethanol Control is stable until the expiration date on the package when stored unopened at 2-8°C and 45 days after opening when stored at 2-8°C. Discard any contaminated material. Microbial contamination is evidenced by an increase in turbidity and/or a characteristic odor.

⚠ PRECAUTIONS

Human source material. Treat as potentially infectious.

Each serum/plasma donor unit used in the manufacture of this product has been tested by FDA accepted methods and found non-reactive for the presence of HBsAg and antibody to HIV-1/2, HCV and HIV-1 Ag. While these methods are highly accurate, they do not guarantee that all infected units will be detected. Because no known test method can offer complete assurance the hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV) or other infectious agents are absent, all products containing human source material should be considered potentially infectious and handled with the same precautions used with patient specimens.

This product contains 0.09% sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form potentially explosive compounds. Flush with excess water upon disposal.

PROCEDURE

Allow the refrigerated controls to warm to room temperature (18-25° C) and gently swirl the control material prior to use in order to ensure product homogeneity. QC materials should be used in accordance with local, state, and/or federal regulations or accreditation requirements.

LIMITATIONS

This material is a control for methods listed in the ASSIGNED VALUES section; it is not to be used as a calibrator. Accurate and reproducible results are dependent upon properly functioning instruments, reagents, standardization, and proper laboratory techniques. Individual laboratories may not obtain the mean assigned value as listed.

VALUE ASSIGNMENT

The mean values and expected ranges printed in this insert were derived from extensive replicate analyses and are specific for this lot.

Values listed below were generated by Cliniqa, the reagent/instrument manufacturer and/or independent laboratories in accordance with an established protocol.

Procedural or assay modifications may alter the mean value obtained. Each laboratory should establish its own parameters of precision; use the mean assigned values and expected ranges provided only as guidelines.

ASSIGNED VALUES

Level 3		Lot No.: 0911121A Exp. Date: 2014-03	
Method	**Units**	**Mean**	**Expected Range**
Gas Chromatography	mg/dL	292	262 - 321

REFERENCES

Baselt, R.C. Analytical Procedures for Therapeutic Drug Monitoring and Emergency Toxicology. Littleton, MA, PSG Publishing, 1987.

IVD **For in vitro diagnostic use**

See package insert for proper use

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P: +31 (0)6 516 536 26

RE-ORDER INFORMATION
Whole Blood Ethanol Control

Catalog No.	Catalog No.	Catalog No.
REF 93211	REF 93212	REF 93213
Level 1, 6 x 5 mL	Level 2, 6 x 5 mL	Level 3, 6 x 5 mL

CLINIQA

lipomed
SERVICES TO HEALTH

Specifications and Certificate of Analysis

Lipomed Document QC-CA-ETH-40-1ML
Version: 001-13 Jan 2012

Supersedes: new

Product name: **40 mg/dL Aqueous Ethanol Standard Solution**
0.040 % by Mass (40 mg Ethanol / 1 dL Water) – 1 ml / ampoule
Ethyl alcohol

Lot Nr: 30112011-B
Art. Nr.: ETH-40-1ML

Release date: 16 01 2012
Expiry date: **November 2016**

Bulk Product Information: Ethanol

Chemical formula:	C_2H_6O	Molwt: 46.07
CAS Registry Nr:	64-17-5	
Purity Ethanol	GC/FID: 100 %	
Water content	Karl Fischer: 0.08 %	

TEST	SPECIFICATIONS	RESULTS
1. Appearance	Clear colorless solution	conforms
2. Identity	GC/FID Headspace R_t corresponds to R_t of NIST reference standard (± 0.10 min)	R_t standard = 1.58 min R_t test = 1.58 min
3. Concentration of calibrated ampoule (GC/FID Headspace)	40.00 ± 0.80 mg/dL	**39.29 ± 0.63 mg/dL** [a] (mean value) (Compared to NIST SRM 2891; 2892; 2893; 2894)
4. Extractable volume	> 1 ml	conforms
5. Water quality	Pharmaceutical water for injection	conforms

a : The concentration of the ampoules is calculated from the distribution of 6 GC/FID Headspace analyses compared with the calibration curve of 2 ampoules of each NIST SRM 2891; 2892; 2893; 2894 with a 95% level of confidence.

During the preparation, the content has been corrected to account for the purity of ethanol and residual water.

FOR ANALYTICAL PURPOSES ONLY: NOT FOR HUMAN OR ANIMAL USE!

Storage conditions: For maximum stability store air-tight below 30 °C in a dark location. Do not freeze.

Note: Gravimetric preparation of each reference solution is ensured by using balances calibrated with ilac-MRA traceable weights.
Lipomed disclaims any liability with respect to mistakes due to inadvertence (e.g. slips of the pen) readily identifiable for an expert or a practitioner.

QC - Officer: Deputy: Dr. L. Prévot

Date of signature: Arlesheim,

January 16, 2012

Prévot

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Ampoule to ampoule consistency:

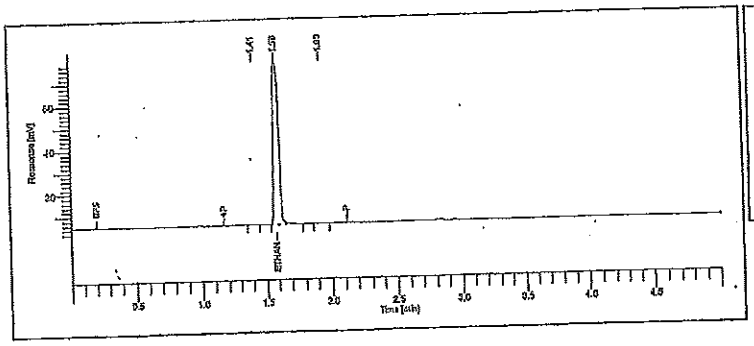
	Specification	Result
% RSD	< 2 %	1.6 %

Homogeneity of the lot is confirmed by an analysis of 6 ampoules. These samples are representative of the batch from which they were taken.

Lot to Lot Consistency:

Standard solution	Lot Number	Concentration
Actual Lot.	30112011-B	39.29 ± 0.63 mg/dL
Previous Lot	N/A	N/A

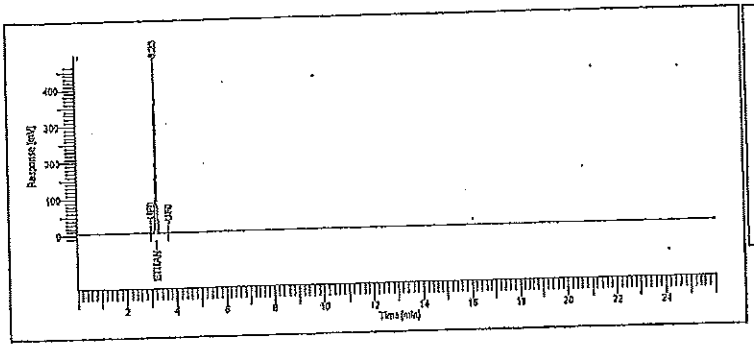
GC/FID Headspace Data: Calibration



Analytical conditions:

column:
Restek BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 15 min

GC/FID Data: Ethanol purity



Analytical conditions:

column:
BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min Isotherm
Helium 100 kPa (GC), 125 kPa (HS)
range 1, attenuation -6
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 25 min

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Specifications and Certificate of Analysis

Lipomed Document QC-CA-ETH-80-1ML
Version: 001-22 Jun 2011

Supersedes: new

Product name: **80 mg/dL Aqueous Ethanol Standard Solution**
0.080 % by Mass (80 mg Ethanol / 1 dL Water) – 1 ml / ampoule
Ethyl alcohol

Lot Nr: 14112011-A
Art. Nr.: ETH-80-1ML

Release date: 26 01 2012
Expiry date: **November 2016**

Bulk Product Information: Ethanol

Chemical formula:	C_2H_6O	Molwt: 46.07
CAS Registry Nr:	64-17-5	
Purity Ethanol	GC/FID: 100 %	
Water content	Karl Fischer: 0.08 %	

TEST	SPECIFICATIONS	RESULTS
1. Appearance	Clear colorless solution	conforms
2. Identity	GC/FID Headspace R_t corresponds to R_t of NIST reference standard (± 0.10 min)	R_t standard = 1.58 min R_t test = 1.58 min
3. Concentration of calibrated ampoule (GC/FID Headspace)	80.00 ± 1.60 mg/dL	79.92 ± 1.37 mg/dL [a] (mean value) (Compared to NIST SRM 2891; 2892; 2893; 2894)
4. Extractable volume	> 1 ml	conforms
5. Water quality	Pharmaceutical water for injection	conforms

a : The concentration of the ampoules is calculated from the distribution of 6 GC/FID Headspace analyses compared with the calibration curve of 2 ampoules of each NIST SRM 2891; 2892; 2893; 2894 with a 95% level of confidence.

During the preparation, the content has been corrected to account for the purity of ethanol and residual water.

FOR ANALYTICAL PURPOSES ONLY: NOT FOR HUMAN OR ANIMAL USE!

Storage conditions: For maximum stability store air-tight below 30 °C in a dark location. Do not freeze.

Note: Gravimetric preparation of each reference solution is ensured by using balances calibrated with ilac-MRA traceable weights.
Lipomed disclaims any liability with respect to mistakes due to inadvertence (e.g. slips of the pen) readily identifiable for an expert or a practitioner.

QC - Officer: Deputy: Dr. L. Prévot

Date of signature: Arlesheim,

January 26, 2012

Prévot

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Ampoule to ampoule consistency:

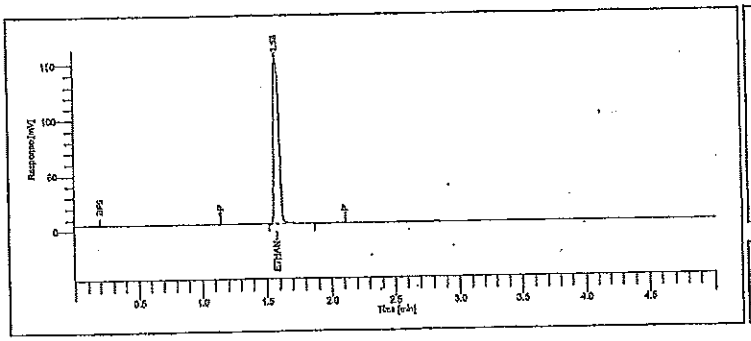
	Specification	Result
% RSD	< 2 %	1.72 %

Homogeneity of the lot is confirmed by an analysis of 6 ampoules. These samples are representative of the batch from which they were taken.

Lot to Lot Consistency:

Standard solution	Lot Number	Concentration
Actual Lot	14112011-A	79.92 ± 1.37 mg/dL
Previous Lot	21022011-A	80.13 ± 0.40 mg/dL

GC/FID Headspace Data: Calibration

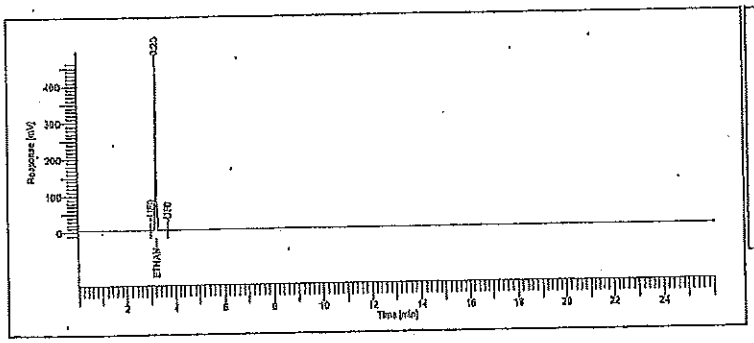


Analytical conditions:

column:
Restek BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 15 min

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]
1	Ethanol	1.58	443327.0	100.000

GC/FID Data: Ethanol purity



Analytical conditions:

column:
BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
range 1, attenuation -6
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 25 min

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Specifications and Certificate of Analysis

Lipomed Document QC-CA-ETH-150-1ML
Version: 001-22 Jun 2011

Supersedes: new

Product name: **150 mg/dL Aqueous Ethanol Standard Solution**
0.150 % by Mass (150 mg Ethanol / 1 dL Water) – 1 ml / ampoule
Ethyl alcohol

Lot Nr: 11012012-C
Art. Nr.: ETH-150-1ML

Release date: 09 02 2012
Expiry date: **January 2017**

Bulk Product Information: Ethanol

Chemical formula:	C_2H_6O	Molwt: 46.07
CAS Registry Nr:	64-17-5	
Purity Ethanol	GC/FID: 100 %	
Water content	Karl Fischer: 0.08 %	

TEST	SPECIFICATIONS	RESULTS
1. Appearance	Clear colorless solution	conforms
2. Identity	GC/FID Headspace R_t corresponds to R_t of NIST reference standard (± 0.10 min)	R_t standard = 1.56 min R_t test = 1.56 min
3. Concentration of calibrated ampoule (GC/FID Headspace)	150.00 ± 3.00 mg/dL	**150.04 ± 1.35 mg/dL** [a] (mean value) (Compared to NIST SRM 2893; 2894; 2895; 2896)
4. Extractable volume	> 1 ml	conforms
5. Water quality	Pharmaceutical water for injection	conforms

a : The concentration of the ampoules is calculated from the distribution of 6 GC/FID Headspace analyses compared with the calibration curve of 2 ampoules of each NIST SRM 2893; 2894; 2895; 2896 with a 95% level of confidence.

During the preparation, the content has been corrected to account for the purity of ethanol and residual water.

FOR ANALYTICAL PURPOSES ONLY: NOT FOR HUMAN OR ANIMAL USE!

Storage conditions: For maximum stability store air-tight below 30 °C in a dark location. Do not freeze.

Note: Gravimetric preparation of each reference solution is ensured by using balances calibrated with ilac-MRA traceable weights.
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QC - Officer: Deputy: Dr. L. Prévot

Date of signature: Arlesheim,

February 09, 2012

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Ampoule to ampoule consistency:

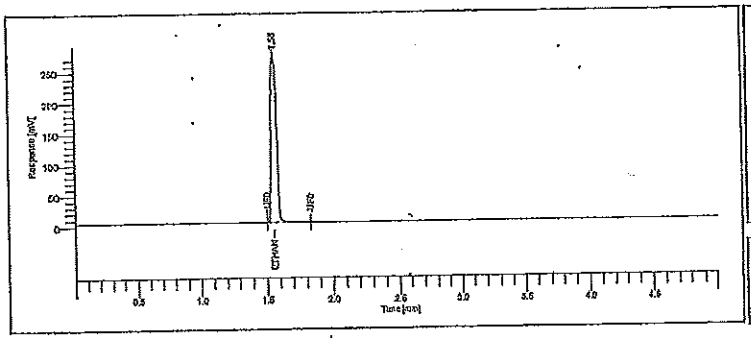
	Specification	Result
% RSD	< 2 %	0.90 %

Homogeneity of the lot is confirmed by an analysis of 6 ampoules. These samples are representative of the batch from which they were taken.

Lot to Lot Consistency:

Standard solution	Lot Number	Concentration
Actual Lot	11012012-C	150.04 ± 1.35 mg/dL
Previous Lot	22022011-A	151.49 ± 0.59 mg/dL

GC/FID Headspace Data: Calibration

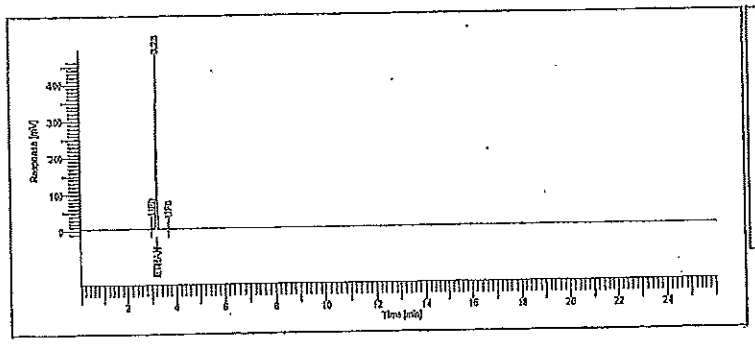


Analytical conditions:

column:
Restek BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 15 min

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]
1	Ethanol	1.56	777173.3	100.000

GC/FID Data: Ethanol purity



Analytical conditions:

column:
BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
range 1, attenuation -6
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 25 min

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Specifications and Certificate of Analysis

Lipomed Document QC-CA-ETH-400-1ML
Version: 001-22 Jun 2011 Supersedes: new

Product name: **400 mg/dL Aqueous Ethanol Standard Solution**
0.400 % by Mass (400 mg Ethanol / 1 dL Water) – 1 ml / ampoule
Ethyl alcohol

Lot Nr: 05012012-C
Art. Nr.: ETH-400-1ML

Release date: 27 01 2012
Expiry date: **January 2017**

Bulk Product information: Ethanol

Chemical formula:	C_2H_6O	Molwt: 46.07
CAS Registry Nr:	64-17-5	
Purity Ethanol	GC/FID: 100 %	
Water content	Karl Fischer: 0.08 %	

TEST	SPECIFICATIONS	RESULTS
1. Appearance	Clear colorless solution	conforms
2. Identity	GC/FID Headspace R_t corresponds to R_t of NIST reference standard (± 0.10 min)	R_t standard = 1.57 min R_t test = 1.57 min
3. Concentration of calibrated ampoule (GC/FID Headspace)	400.00 ± 8.00 mg/dL	**400.73 ± 3.31 mg/dL** [a] (mean value) (Compared to NIST SRM 2893; 2894; 2895; 2896)
4. Extractable volume	> 1 ml	conforms
5. Water quality	Pharmaceutical water for injection	conforms

a : The concentration of the ampoules is calculated from the distribution of 6 GC/FID Headspace analyses compared with the calibration curve of 2 ampoules of each NIST SRM 2893; 2894; 2895; 2896 with a 95% level of confidence.

During the preparation, the content has been corrected to account for the purity of ethanol and residual water.

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QC - Officer: Deputy: Dr. L. Prévot

Date of signature: Arlesheim,

January 27, 2012

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Ampoule to ampoule consistency:

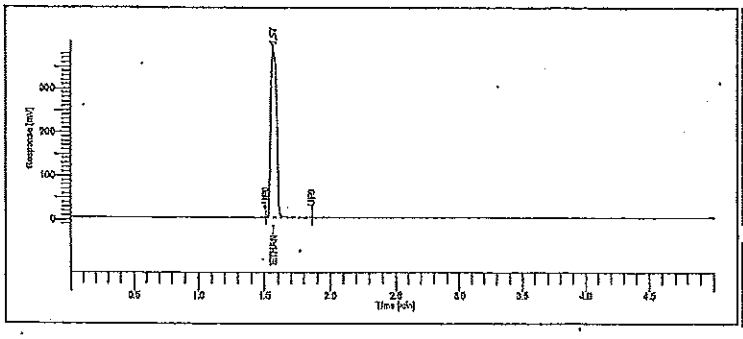
	Specification	Result
% RSD	< 2 %	0.83 %

Homogeneity of the lot is confirmed by an analysis of 6 ampoules. These samples are representative of the batch from which they were taken.

Lot to Lot Consistency:

Standard solution	Lot Number	Concentration
Actual Lot	05012012-C	400.73 ± 3.31 mg/dL
Previous Lot	09032011-G	402.89 ± 4.86 mg/dL

GC/FID Headspace Data: Calibration

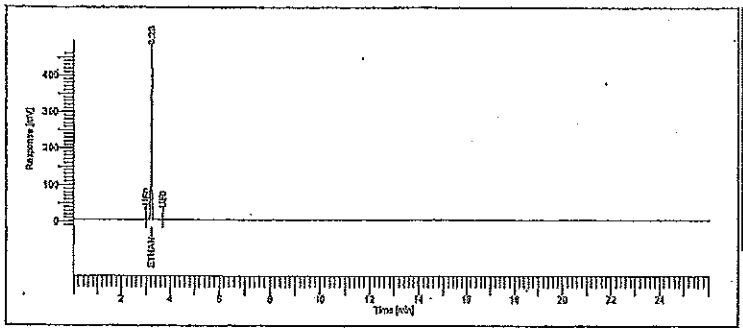


Analytical conditions:

column:
Restek BAC 1, 30 m x 0.32 mm, 1.8 um
Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
pressurization time: 2 min
injection time: 0.05 min
withdrawal time: 0.5 min
needle: 75 °C
transferline: 150 °C
Thermostatisierung: 60 °C, 15 min

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]
1	Ethanol	1.57	1122371.7	100.000

GC/FID Data: Ethanol purity



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column:
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Injektor: 200 °C, split 20 ml/min
FID: 300 °C
Ofen:40 °C, 5 min isotherm
Helium 100 kPa (GC), 125 kPa (HS)
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