CITY OF SAN DIEGO
MEMORANDUM

DATE: April 24, 2024

TO: Shawn Montpetit, Quality Assurance Manager

FROM: Stephanie Lambert, Technical Lead, Forensic Chemistry Unit

SUBJECT: Validation of Perkin Elmer Dual Column Headspace Gas Chromatograph

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The Perkin Elmer Clarus 690 Gas Chromatograph with Turbomatrix Headspace Sampler was purchased to qualitatively and quantitatively analyze ethanol in antemortem blood samples. The columns are equivalent to Elite BAC 1 and Elite BAC 2, here on referred to as BAC 1 and BAC 2, respectively. The BAC 1 column is used for both qualitative and quantitative determinations, with the BAC 2 column being used for qualitative confirmation. Each sample injection is split and run simultaneously on each column. The instrumental method developed is an internal standard method which is able to separate ethanol and the n-propanol internal standard from other common volatiles that can be found in the blood samples. It accurately and precisely measures ethanol and does not detect ethanol when none is present. NIST traceable standards were used along with ethanol positive blood samples. Preparation of calibrators, controls, and samples followed the previously validated procedure to include allowing them to acclimate to room temperature prior to sampling, rocking blood samples, and using a 1:21 dilution of the calibrator, control, or sample to internal standard solution using a calibrated dilutor.

Training

Perkin Elmer engineers and technicians provided training for the Forensic Chemistry Unit on the instrument and software. Each of the three analysts who were competent on the previous Perkin Elmer system for blood alcohol analysis participated in the validation testing and are included in the estimated uncertainty of measurement.

Interferents

Volatile substances that can commonly be found in antemortem blood samples were able to be separated from ethanol and n-propanol on each column. The retention times of more than 20 replicate runs of methanol, acetone, isopropanol, acetaldehyde, and n-propanol were used to establish a retention time window for each substance. The runs showed consistent separation and did not vary more than 1% from the average.

Ethyl chloride and sevoflurane were also evaluated for separation from ethanol and n-propanol and showed consistent separation in five replicate runs.

Calibration

NIST traceable Certified Reference Materials (CRMs) of 0.020, 0.100, 0.200, 0.300, and 0.500 g/100ml were used to establish calibration curves. Each CRM was run one time per line with linear regression, and without forcing through zero. The coefficient of determination, r^2, of each line was required to be at least 0.99. The average of over 20 runs was 0.99993. Each calibrator was reevaluated against the newly created line and 99% of the time the calculated value was within 10% of the expected value.

Accuracy and Precision

The accuracy and precision of the method was evaluated intrarun and interrun, as well as near the lower and upper limits of quantitation. The method was also assessed for any matrix effects. Following successful calibration, the following CRM controls were run: 0.050[2], 0.080, 0.150, and 0.400[2] g/100ml in sets of five. Six batches were run on different days with all analysts approved for BAC casework participating. Additionally, the 0.050, 0.080, 0.150, and 0.400 g/100 mL controls were run after the calibration of the instrument for all other validation runs. The accuracy of the controls were within 0.005 g/100ml of the expected value for values under 0.100 g/100ml and within 5% of the expected value for values of 0.100 g/100ml or more[3]. The overall coefficient of variation for each level was less than 2.5%[1].

An ethanol positive whole blood control was run to assess the accuracy and precision of the method on alcohol positive blood samples. This control was run in sets of five. Six batches were run on different days with all analysts approved for BAC casework participating. The overall coefficient of variation for the whole blood control was less than 3%.

Duplicates of four different CTS blood samples were run and the results were compared to the Grand Means reported by CTS. All results were within three standard deviations of their reported Grand Mean.

Limit of Quantitation

The upper and lower limits of quantitation have been administratively set as the highest and lowest calibrators, respectively[1]. Controls run at both the low and high ends of the range confirmed linearity throughout the range.

Limit of Detection

The lowest concentration CRM, 0.010 g/100ml, was run a minimum of twice per analyst, with no more than two runs per day, to assess the ability of the instrument and method to reliably detect low levels of ethanol. The method was not required to properly quantitate this level of ethanol but had to detect and integrate it each time. With the exception of the first two sets of runs, the 0.010 g/100 mL standard was reliably detected on both columns. The initial runs showed detection with column one but not with column two. The peak detection parameters were adjusted immediately following those runs and the issue was resolved. Ethanol negative samples produced a value of 0.0000 g/100 mL[3] each time they were run.

Internal Standard Recovery

The internal standard recovery was assessed for each control and sample in runs 1.1-1.5 and 2.1-4.1, accounting for over 500 data points. For each run, the areas under the

internal standard curves for the five calibrators were averaged. This averaged value was then set as 100% recovery. The area under the internal standard curve of each control and sample was compared to this value and the difference was calculated as a percentage. The maximum difference observed was less than 11%.

The average recovery was 98.7% with a standard deviation of 2.9%. 94.5% of the recoveries fell within two standard deviations and 99.5% fell within three standard deviations.

Carryover

Carryover was assessed two different ways. A negative control was run immediately after the 0.500 g/100 mL CRM in each run to assess the possibility of carryover from high levels of ethanol. The negative control returned a result of 0.0000 g/100 mL of ethanol in every run.

The second assessment of carryover was to show that there is no additive effect of running multiple alcohol positive samples in succession. This was assessed by "filling the wheel" with ethanol positive blood samples from the same blood tube. After successful calibration, 93 ethanol positive vials were run, followed by a negative control and two additional positive controls (0.08 and 0.15 g/100mL). The average and standard deviation of the ethanol positive samples were determined for each run. Greater than 99% of the results were expected to fall within three standard deviations of the average, no trends were expected, and all final negative controls were expected to result in 0.0000 g/100 mL. While no trends were detected and no ethanol carried over into the negative controls, the results of two of the three runs showed that in less than 50 injections, more than the expected number of samples were outside of the range of three standard deviations. It was determined that there was a difference in the sampling of the replicate blood samples as compared to casework and the sampling procedure was adjusted. Five additional runs were completed to address the issues. Each run was comprised of a standard calibration curve and set of controls. Three runs included 50 replicate bloods each sampled as casework, one run included 50 replicates of a 0.0500 g/100 mL standard, and one run included 10 replicates each of the 0.0800 and 0.0150 g/100 mL standards spread across a run of the full wheel. The additional testing showed all samples within three standard deviations of the expected values and in line with percent CV values seen with replicate control testing.

Stability of Ethanol Level

The stability of ethanol in blood samples stored in gray top tubes is well established in literature and has previously been confirmed in house with the rerunning of adjudicated samples. It was not assessed again during this validation since it is not method or instrument dependent.

The stability of the ethanol in headspace vials prepared one day and run another was assessed by comparing data from samples stored overnight at room temperature, samples stored overnight in the refrigerator, and samples prepared the day they were run. The refrigerated samples spent approximately 24 hours in refrigeration. Both sets of samples stored overnight were run within 36 hours of preparation. Two ethanol positive blood samples, with blood alcohol contents of approximately 0.061 g/100 mL and 0.261 g/100 mL respectively, were selected for analysis. Two sets of calibrators and controls, and two sets of duplicate ethanol positive samples were prepared consecutively. One set of calibrators and controls and one set of samples were kept at room temperature overnight and the second set

of each was placed in the refrigerator overnight. The following day a set of calibrators, controls, and duplicate ethanol positive samples (from the same tubes used the day before) were prepared. Each batch was run and the results were compared. Ethanol positive samples for each level were within 3.5% of each other across preparation parameters. This procedure was repeated twice by two different analysts on two different days.

Uncertainty of Measurement

Upon completion of the validation, the results from throughout were used to assist in determining the uncertainty of measurement of this method for the quantitation of ethanol in blood. Per Title 17, the uncertainty of measurement must fall within 0.005 g/100 mL of the expected value for values under 0.100 g/100 mL, and within 5% of the expected value for values of 0.100 g/100 mL or more[3] in order to be deemed acceptable for casework. This method performed within those parameters. The full uncertainty of measurement write-up can be found as a separate document.

Summary of Results

The instrumental method accurately and precisely quantitates ethanol from 0.020 g/100 mL to 0.500 g/100 mL. The BAC 1 column is used for both qualitative and quantitative determinations, while the BAC 2 column is used for qualitative confirmation. The method separates ethanol and the n-propanol internal standard from other common volatiles that can be found in the blood samples and does not detect ethanol when none is present. The reproducibility is well within accepted limits as dictated by Title 17[3].

The totality of the results produced through this validation process demonstrate that the dual column Perkin Elmer Clarus 690 Gas Chromatograph with Turbomatrix Headspace Sampler and method are suitable for use in antemortem blood alcohol testing.

References

(1) *SOFT/AAFS Forensic Toxicology Laboratory Guidelines*, 2006 Version.
(2) *Standard for a Quality Control Program in Forensic Toxicology Laboratories*, ASB Standard 054, 2020 Draft.
(3) *Forensic Alcohol Analysis and Breath Alcohol Analysis*, California Code of Regulations Title 17, 2017 Revision.

Technical lead _____ Date 4/26/24

Supervisor _____ Date 4/26/24

SDPD Forensic Chemistry Section
Forensic Alcohol Analysis – Establishment of the Uncertainty of Measurement of Ethanol in Blood
Headspace Gas Chromatography - GC6

Introduction

This is the initial establishment of the Uncertainty of Measurement (UM) for GC6, a Perkin Elmer Clarus 690 GC/FID with a Turbomatrix 110 heated headspace autosampler. Sources of uncertainty were determined to include the instrument, the sample diluter, analysts, certified reference materials, and environmental conditions that could vary by day and time of day. The data collected incorporates over 200 control runs covering three analysts, various room temperatures, multiple days, and multiple times of day.

Procedure

Each run consisted of certified reference material (CRM) calibrators of 0.0200 g/100 mL, 0.1000 g/100 mL, 0.2000 g/100 mL, 0.3000 g/100 mL, and 0.5000 g/100 mL. After the calibration line, 0.0500 g/100 mL, 0.0800 g/100 mL, 0.1500 g/100 mL, and 0.4000 g/100 mL CRM controls were run. Additional 0.0800 g/100 mL and 0.1500 g/100 mL CRM controls were run during some tests and were also included in the calculations.

Defining Factors of Uncertainty of Measurement

In estimating measurement uncertainty, the following factors that affect the measurement were assessed:

- **Reported Accuracy of Certified Reference Material Ethanol Standards:**
  The standards are CRMs with values traceable to NIST Standardized Reference Material. Since various lots were utilized, the following are the highest manufacturer established uncertainty values at a k = 1.

  1. 0.0200 g/100 mL: ± 0.00004 g/100 mL
  2. 0.0500 g/100 mL: ± 0.000095 g/100 mL
  3. 0.0800 g/100 mL: ± 0.0002 g/100 mL
  4. 0.1000 g/100 mL: ± 0.0002 g/100 mL
  5. 0.1500 g/100 mL: ± 0.0005 g/100 mL
  6. 0.2000 g/100 mL: ± 0.0004 g/100 mL
  7. 0.3000 g/100 mL: ± 0.0006 g/100 mL
  8. 0.4000 g/100 mL: ± 0.0008 g/100 mL
  9. 0.5000 g/100 mL: ± 0.001 g/100 mL

- **Accuracy and Linearity:**
  The instrument's ability to measure ethanol levels accurately and throughout a range of values represents the accuracy and linearity. The average difference of each of the four CRM control levels was calculated to determine accuracy and linearity.

- **Precision (repeatability)**
  The instrument's ability to consistently deliver the same reading of a known amount represents precision. The standard deviation of each of the four CRM control levels was calculated to determine precision.

- **The sample diluter and the Headspace GC/FID instrument:**
  The uncertainty associated with these aspects of sample preparation and quantitation is accounted for in the results collected and is reflected in the measurements of accuracy and precision. These were not separately included into the overall expanded uncertainty calculations.

Calculating the Uncertainty of Measurement

The validation data was analyzed to calculate the standard deviation (repeatability) and average difference (accuracy and linearity) for each control level. A normal distribution was assumed, and standard uncertainties were calculated and combined. The combined uncertainty for each level was calculated at a coverage factor of k = 2. Table 1 summarizes these calculations for 0.0500 and 0.0800 g/100 mL. Table 2 summarizes these calculations for 0.1500 and 0.4000 g/100 mL. See Appendix A for calculation sheets.

Table 1

g/100 mL	Factor	Value (x)	Std U (u)	Combined UM	k = 2	Percent at k = 2
0.0500	Repeatability[1]	0.001082	0.001082	0.001094	0.0022	4.4%
	Accuracy and Linearity[2]	0.000129	0.000129			
	Standards (highest)	0.000095	0.000095			
0.0800	Repeatability[1]	0.001471	0.001471	0.001563	0.0031	3.9%
	Accuracy and Linearity[2]	0.000490	0.000490			
	Standards (highest)	0.000200	0.000200			

Table 2

g/100 mL	Factor	Value (x)	Std U (u)	Combined UM	k = 2	Percent at k = 2
0.1500	Repeatability[1]	0.001913	0.001913	0.002109	0.0042	2.8%
	Accuracy and Linearity[2]	0.000733	0.000733			
	Standards (highest)	0.000500	0.000500			
0.4000	Repeatability[1]	0.003804	0.003804	0.003891	0.0078	2.0%
	Accuracy and Linearity[2]	0.000177	0.000177			
	Standards (highest)	0.000800	0.000800			

Reporting the Uncertainty of Measurement

The highest combined uncertainty under a 0.100 g/100 mL was determined to be +/-0.003 g/100 mL at the 0.080 g/100 mL level. The highest combined uncertainty at or above a 0.100 g/100 mL was determined to be +/-2.8% at the 0.150 g/100 mL level.

For values from 0.020 g/100 mL up to 0.100 g/100 mL, the uncertainty will be expressed as +/- 0.003 g/100 mL. For values at or above 0.100 g/100 mL up to 0.500 g/100 mL, the uncertainty will be expressed as +/-2.8%. These uncertainty of measurement values are at a coverage level of k = 2, a confidence level of approximately 95%, and are within the acceptable limits set by Title 17. Values below 0.020 g/100 mL or above 0.500 g/100 mL will not have an associated UM as they are outside of the lower and upper limits of quantitation.

Reevaluating Uncertainty of Measurement

The uncertainty of measurement will be reevaluated whenever any part of the process is altered that will affect the measurement quantitation. This may include repair or upgrade of the GC or headspace instruments, repairing or replacing the sample diluter, or changes to the quantitation method. Additionally, prior to a new analyst being approved for casework in blood alcohol analysis, they will be required to run controls as listed in the manual, calculate their uncertainty contributions, and ensure that they are within the established limits. If, during a reevaluation, the UM is found to have changed, the UM will be reestablished.

Control charts will be used to monitor the UM over time, with reestablishments being done as deemed necessary.

Analyst _____ Date 4/26/24

Technical Review _____ Date 04/26/24

Administrative Review _____ Date 4/26/24

Headspace GC6
Under 0.1000%
Acceptable Range: +/- 0.005ǂ

g/100 mL	Factor	Value (x)	Standard Uncertainty (u)	Distribution	Relative Contribution*
0.0500	Repeatability[1]	0.001082	0.001082	Normal	83%
	Accuracy and Linearity[2]	0.000129	0.000129	Normal	10%
	Standards (highest)	0.000095	0.000095	Normal	7%
0.0800	Repeatability[1]	0.001471	0.001471	Normal	68%
	Accuracy and Linearity[2]	0.000490	0.000490	Normal	23%
	Standards (highest)	0.000200	0.000200	Normal	9%

ǂAs per Title 17
[1]Standard deviation
[2]Average difference
*Each standard uncertainty value divided by the total standard uncertainty multiplied by 100.

Calculation of Combined Standard Uncertainty:

$$U_c = \sqrt{u(repeatability)^2 + u(accuracy\ and\ linearity)^2 + u(standard)^2}$$

Calculation of Expanded Uncertainty:

U = k × *Uc*
U is the expanded uncertainty
k is the coverage factor (approximately 95% when k = 2)

0.0500%

Calculation of Combined Standard Uncertainty:

$$U_c = \sqrt{u(0.001082)^2 + (0.000129)^2 + (0.000095)^2} = 0.001094$$

Calculation of Expanded Uncertainty:

$$U = 2 * 0.001094 = 0.0022$$

0.0800%

Calculation of Combined Standard Uncertainty:

$$U_c = \sqrt{u(0.001471)^2 + (0.00490)^2 + (0.000200)^2} = 0.001563$$

Calculation of Expanded Uncertainty:

$$U = 2 * 0.001563 = 0.0031$$

The highest calculated expanded uncertainty is within the acceptable range as defined by Title 17, +/- 0.005 g/100 mL.

Analyst _____ Date 4/26/24

Technical Review _____ Date 042624

Administrative Review _____ Date 4/26/24

Headspace GC6
0.1000% and Over
Acceptable Range: +/- 5%[†]

g/100 mL	Factor	Value (x)	Standard Uncertainty (u)	Distribution	Relative Contribution*
0.1500	Repeatability[1]	0.001913	0.001913	Normal	61%
	Accuracy and Linearity[2]	0.000733	0.000733	Normal	23%
	Standards (highest)	0.000500	0.000500	Normal	16%
0.4000	Repeatability[1]	0.003804	0.003804	Normal	80%
	Accuracy and Linearity[2]	0.000177	0.000177	Normal	4%
	Standards (highest)	0.000800	0.000800	Normal	17%

[†]As per Title 17
[1]Standard deviation
[2]Average difference
*Each standard uncertainty value divided by the total standard uncertainty multiplied by 100.

Calculation of Combined Standard Uncertainty (0.200):

$$U_c = \sqrt{u(repeatability)^2 + u(accuracy\ and\ linearity)^2 + u(standard)^2}$$

Calculation of Expanded Uncertainty:

U = k × *Uc*
U is the expanded uncertainty
k is the coverage factor (approximately 95% when k = 2)

0.1500%

Calculation of Combined Standard Uncertainty:

$$U_c = \sqrt{u(0.001913)^2 + (0.000733)^2 + (0.0005)^2} = 0.002109$$

Calculation of Expanded Uncertainty:

$$U = 2 * 0.002109 = 0.0042 = 2.8\%$$

0.4000%

Calculation of Combined Standard Uncertainty:

$$U_c = \sqrt{u(0.003804)^2 + (0.000177)^2 + (0.000800)^2} = 0.003891$$

Calculation of Expanded Uncertainty:

$$U = 2 * 0.003891 = 0.0078 = 2.0\%$$

The highest calculated expanded uncertainty is within the acceptable range as defined by Title 17, +/- 5%.

Analyst _____ Date 4/26/24

Technical Review _____ Date 04/26/24

Administrative Review _____ Date 4/26/24