CITY OF SAN DIEGO
MEMORANDUM

DATE: August 28, 2024

TO: Shawn Montpetit, Quality Assurance Manager

FROM: Stephanie Lambert, Technical Lead, Forensic Chemistry Unit

SUBJECT: Verification of The Perkin Elmer Clarus 590 Gas Chromatograph with Turbomatrix Headspace Sampler (GC5)

The Perkin Elmer Clarus 590 Gas Chromatograph with Turbomatrix Headspace Sampler (GC5) was purchased to qualitatively and quantitatively analyze ethanol in antemortem blood samples. The columns are equivalent to Elite BAC 1 and Elite BAC 2, hereon referred to as BAC1 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 the presence of ethanol when none is present. NIST traceable standards were used along with ethanol positive blood samples. Preparation of calibrators, controls, and samples followed the currently validated procedure to include allowing them to acclimate to room temperature prior to sampling and using a 1:21 dilution of the calibrator, control, or sample to internal standard solution using a calibrated dilutor.

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 over a dozen replicate runs of methanol, ethanol, acetone, isopropanol, acetaldehyde, and n-propanol were used to establish a retention time window for that substance. The runs showed consistent separation and not vary more than 2% from the average.

The separation of ethyl chloride and sevoflurane from ethanol and n-propanol was also confirmed.

Calibration

NIST traceable Certified Reference Materials (CRMs) at levels of 0.020, 0.100, 0.200, 0.300, and 0.500 g/100 mL 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 the line was required to be at least 0.99 for the line to be acceptable.

The average for over a dozen runs was 0.999965. Each calibrator was then reevaluated against the newly created line and 100% 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 both 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, 0.080, 0.150, and 0.400 g/100 mL in duplicates. 13 batches were run over 11 days, with no more than two runs per day, and with all analysts approved for BAC casework participating. The accuracy of the controls were 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. The overall coefficient of variation for each level was less than 1%.

An ethanol positive whole blood control was also run to assess the accuracy and precision of the method on alcohol positive blood samples. This control was run in duplicates over 10 days, with no more than two runs per day, and with all analysts approved for BAC casework participating. The overall coefficient of variation for the whole blood control was less than 1%.

Limit of Quantitation

The upper and lower limits of quantitation have been administratively set as the highest and lowest calibrators, respectively. 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/100 mL, was run at least once per analyst, with no more than two runs per day, to assess the ability of the method to reliably detect low levels of ethanol. The method was not required to properly quantitate this level of ethanol but must detect and integrate it each time. Ethanol negative samples produced a value of 0.0000 g/100 mL each time they were run.

Internal Standard Recovery

The internal standard recovery was assessed for each run. 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 6%.

Carryover

Carryover was assessed by running a negative control 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 0.000 g/100 mL of ethanol in every run.

Uncertainty of Measurements

Upon completion of the verification, 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 measurements 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 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 the accepted limits as dictated by Title 17.

The totality of the results produced through this verification process demonstrate that the dual column Perkin Elmer Clarus 590 Gas Chromatograph with Turbomatrix Headspace Sampler and method are suitable for use in antemortem blood alcohol testing.

Technical Lead _____ Date 8/28/24

Supervisor _____ Date 9/17/24