SDPD Forensic Chemistry Section
Forensic Alcohol Analysis - Uncertainty of Measurement of Ethanol in Blood and Urine
By Headspace Gas Chromatography

Introduction

For this study, a Perkin Elmer Clarus 500 Gas Chromatograph (HSGC3) coupled with a flame ionization detector, a heated headspace autosampler Turbomatrix 110, and a gas chromatography data handling system was utilized. To estimate uncertainty of measurement, the process of alcohol quantitation was evaluated to include the following sources of uncertainty: HSGC3 instrument, the sample diluter, different analysts, different days, and different times of day.

Procedure

Each analyst ran at least once on HSGC3 to include ten replicates of the 0.050g%, 0.080g%, 0.200g%, and the 0.300g% standards, and two replicates of the 0.400g% standard. Each run was performed on different days and at different times of the day.

Defining Factors of Uncertainty of Measurement

In estimating measurement uncertainty, the following factors that affect the measurement taken will be addressed:

- **Reported Accuracy of Certified Reference Material (CRM) Ethanol Standards:**
  The standards are Certified Reference Materials (CRM) with values traceable to NIST Standardized Reference Material (SRM). Five (5) different levels of ethanol were analyzed with the following manufacturer established values (± margin of error is for a 95% confidence interval).

  1. **0.050g% Standard (± 0.00018)**
  2. **0.080g% Standard (± 0.00028)**
  3. **0.200g% Standard (± 0.00070)**
  4. **0.300g% Standard (± 0.00106)**
  5. **0.400g% Standard (± 0.00140)**

- **Accuracy and Linearity:**
  The instruments' ability to measure ethanol levels accurately and throughout a range of values represents the accuracy and linearity. The average difference of each of the five (5) CRM standard levels will be calculated to determine accuracy and linearity. This measurement encompasses the intra-analyst variability, inter-analyst variability, time of day variability, and different day variability although there were no significant differences found.

- **Precision (repeatability)**
  The instruments ability to consistently deliver the same reading of a known amount. The standard deviation of repeated measurements was calculated and is a statistical measure of precision.

- **The sample diluter and the Headspace GC/FID instrument:**
  Variability associated with these aspects of sample preparation and quantitation are incorporated into our evaluation of the whole process of alcohol quantitation and will be reflected in the measurements of accuracy and precision.

- **Type A Uncertainties:** Uncertainty results from a measurement that are scattered in a random fashion due to chance. This uncertainty can be determined by a large number of repeated measurements and is considered to be a normal or Gaussian shaped distribution.
  - Precision (Repeatability)
  - Accuracy and Linearity

- **Type B Uncertainties:** Systematic inherent bias in the quantitative analytical process. This uncertainty is considered to be a uniform or rectangular distribution, with each potential value measurement bias being equally likely over an interval.
  - Accuracy of Certified Reference Material ethanol values
    The manufacturer's reported uncertainty is not a significant contributor to the overall expanded uncertainty and will not be incorporated into the calculation.

Reporting the Uncertainty of Measurement

Reporting the uncertainty for values under 0.100g% will be expressed as +/-0.005g% as per Title 17. Reporting the uncertainty for values greater than or equal to 0.100g% will be expressed as a percentage at a coverage level of k = 2 and a confidence level of approximately 95%.

The maximum combined uncertainty was calculated at a coverage factor of k=2 for values under 0.100g% using the maximum standard deviation and the maximum average difference. This value must be less than 0.005g%, as per Title 17.

For values of 0.100g% or greater, the combined uncertainty was calculated at a coverage factor of k=2 for each standard. The uncertainties were converted to percentages and the highest value will be used for reporting.

Results:

Table 1 summarizes the uncertainty of measurement results for precision, accuracy, and linearity.

Table 1

CRM Established Value (g%)	Standard Deviation	Average Difference
0.050	0.00054	0.00119
0.080	0.00058	0.00113
0.200	0.00160	0.00160
0.300	0.00234	0.00136
0.400	0.00233	0.00107

Headspace GC3
Under 0.100g%
Acceptable Range: +/- 0.005g%[†]

Factors	Value (x)	Standard Uncertainty (u)	Distribution	Relative contribution*
Repeatability[1]	0.00058	0.00058	Normal	33%
Accuracy and Linearity[2]	0.00119	0.00119	Normal	67%

[†]As per Title 17

[1]Maximum standard deviation (standards 0.050g% and 0.080g%)

[2]Maximum average difference (standards 0.050g% and 0.080g%)

*Each standard uncertainty value divided by the total standard uncertainty multiplied by 100.

Calculation of Combined Standard Uncertainty:

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

$$U_c = \sqrt{(0.00058)^2 + (0.00119)^2} = \mathbf{0.001g\%}$$

Calculation of Expanded Uncertainty:

$U=k\times Uc$ Where U is the expanded uncertainty and k is the coverage factor.

Approximate 95% where k=2 +/- 0.002g%

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

Headspace GC3
0.100g% and Over
Determined acceptable Range: +/- 2.3%

Control (g%)	Factor	Value (x)	Standard Uncertainty (u)	Distribution	Relative Contribution*
0.200	Repeatability[1]	0.00160	0.00160	Normal	50%
	Accuracy and Linearity[2]	0.00160	0.00160	Normal	50%
0.300	Repeatability[1]	0.00234	0.00234	Normal	63%
	Accuracy and Linearity[2]	0.00136	0.00136	Normal	37%
0.400	Repeatability[1]	0.00233	0.00233	Normal	69%
	Accuracy and Linearity[2]	0.00107	0.00107	Normal	31%

[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):

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

$$U_c = \sqrt{(0.00160\ )^2 + (0.00160\ )^2} = \mathbf{0.0022g\%}$$

Calculation of Expanded Uncertainty:

U=k×Uc Where U is the expanded uncertainty and k is the coverage factor.

Approximate 95% where k=2 +/- 0.0044g% => 2.2% (without the truncation of the k=1 value, k=2 is 2.3%. This value (2.3%) will be applied as the limit for values of 0.100 or greater to ensure full coverage)

Calculation of Combined Standard Uncertainty (0.300):

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

$$U_c = \sqrt{(0.00234\ )^2 + (0.00136\ )^2} = \mathbf{0.0027g\%}$$

Calculation of Expanded Uncertainty:

U=k×Uc Where U is the expanded uncertainty and k is the coverage factor.

Approximate 95% where k=2 +/- 0.0054g% => 1.8%

Calculation of Combined Standard Uncertainty (0.400):

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

$$U_c = \sqrt{(0.00233)^2 + (0.00107)^2} = \mathbf{0.0025g\%}$$

Calculation of Expanded Uncertainty:

U=k×Uc Where U is the expanded uncertainty and k is the coverage factor.

Approximate 95% where k=2 +/- 0.0050g% => 1.25%

Reevaluating Uncertainty of Measurement

This study was performed during the following weeks of 2016: January 19th, February 1st, and February 10th, and the week of February 7, 2017. The uncertainty of measurement will be re-established whenever any part of the process is altered so that it will affect the measurement quantitation. This will include repair or upgrade of GC headspace instruments with new hardware or new software that would affect quantitation, and repairing or replacing the sample diluter. Additionally, prior to a new analyst being signed off in blood alcohol analysis, they will be required to run the controls as listed, calculate the uncertainties, and ensure that they are within the established limits.

Technical Review _____ Date 4-6-17

Administrative Review _____ Date 4-6-17

Quality Assurance Manager _____ Date 4/11/17