Title: Procedures for Estimating Measurement Uncertainty (CAS-04)

1. Purpose

Measurement uncertainty calculations are used within quantitative measurement results and are based on certain information that has been acquired about the measurement method for a particular analytical instrument or method. This document describes the approach within the Chemical Analysis Section (CAS) for estimating measurement uncertainties. It is based on a simplified version of the "Guide to the Expression of Uncertainty in Measurement" (or the 'GUM') which is a widely-accepted method for evaluating, estimating, and expressing measurement uncertainty. Another valuable reference is the 8-Step Process found within the National Institute of Standards and Technology (NIST).

2. Scope

Measurement uncertainty values will be evaluated [or estimated], when applicable, for all reported quantitative results. Item descriptors that include numbers (e.g., when describing/listing evidence) are not considered part of the results and therefore do not need uncertainty values calculated for them. Values associated to measurement uncertainty can be, but may not be limited to: values relevant to the validity or interpretation of an examination result, when the value is requested by the customer, or when the measurement value is related to a specific limit or statute level.

This document applies to standard operating procedures within the Chemical Analysis Section in which a quantitative measurement is reported:

- The quantity (mass or volume) of evidence (e.g., a potential controlled substance) when it is reported. Residue weights within the Controlled Substances Unit are not typically reported. Quantities listed in order to describe evidence (e.g., number of pills within a submission or number of tubes of blood) will not be subject to measurement uncertainty.
- The concentration (mass or volume fraction) of a drug in a toxicology sample, including values reported for blood alcohol. Measurement uncertainty will not be calculated nor reported for values reported wherein measurements were conducted from outside laboratories and wherein mathmetical calculations are applied (e.g., serum-ethanol conversions).

Additionally, this document will apply to examinations and subsequent reported results where the estimation of uncertainty is requested by the submitting agency or expressed in jurisdictional or statute requirements.

3. Responsibilities

This document applies to section personnel who report, or assist in the reporting, of measurement results within a laboratory report according to relevant accreditation guidelines.

4. Records

Supporting documentation related to measurement uncertainty estimation will be maintained. Such information may be recorded in multiple locations such as: electronic data within servers (e.g., spreadsheets), standard operating procedures (SOPs), validation binders, and case files. Types of information within such locations can include:

- Statement defining the quantity intended to be measured (i.e., measurand)
- Statement of how traceability is established for the measurement
- The equipment used as measuring devices (i.e., instruments used)
- All applicable parts of measurement processes which may significantly contribute to uncertainty
- Select measurements (e.g., repeatability, reproducibility, precision)
- Calculations used in the process
- Combined uncertainty, coverage factor (*k*), confidence level (also known as the coverage probability), and finally the expanded measurement uncertainty
- The schedule to review and/or recalculate the measurement uncertainty

5. Estimating Measurement Uncertainty

The eight (8) steps listed below are considered a simpliefied approach to the GUM and are used within the CAS to estimate measurement uncertainty:

- Step 1: Define what is being measured and/or specify the measurement process
- Step 2: Evaluate and list sources of uncertainty
- Step 3: Quantify sources of uncertainty
- Step 4: Obtain standard uncertainty values
- Step 5: Calculate combined standard uncertainty
- Step 6: Expand the combined standard uncertainty by coverage factor (*k*)

- Step 7: Express the expanded measurement uncertainty
- Step 8: Report result with the associated uncertainty

The CAS utilizes uncertainty budgets for performing estimation of measurement uncertainty. An example of a spreadsheet used for such calculations can be found within the Appendix. Below is an example of a generic template for starting a measurement uncertainty calculation within the section.

Analyses by Instrumental Method		
Uncertainty for Analyte/Group of Analytes		
Name of Unit		**[From Data Date] to [To Data Date]**
Items that influence method uncertainty (considered in determining uncertainty)		
Influence #1	# %	Explanation of how the value (# %) was obtained.
Influence #2	# %	Explanation of how the value (# %) was obtained.
…	…	…
Instrument where data is collected from reference standards	=MAX(%CV) (From spreadsheet data)	Largest empirical Relative Standard Deviation (% CV) value selected from reference standards (see attached table which lists data from spreadsheet)
Absolute (Ave. Bias)	=MAX (Ave. |Bias|) (From spreadsheet data)	Largest average value indicating degree of accuracy. Obtained by replicate measurements of a reference material. [ABS(Empirical - Theoretical)) / (Theoretical)] x 100

Sources of Uncertainty	Value	Distribution	Divisor based on distribution	Standard Uncertainty (μ_i) (value/distribution)	Relative Index (% factor contributes to the standard uncertainty) ($\mu_i/(\sum\mu_i)$)*100
Influence #1	# %	rectangular	√3	Value (# %) / SQRT(3)	Std. Uncertainty / Sum of Values
Influence #2	# %	rectangular	2*√3 ("2" needed for k=2 to be k=1)	Value (# %) / (2*SQRT(3)	Std. Uncertainty / Sum of Values
…	…	…	…	…	…
Instrument where data is collected from reference standards	# %	Normal	1	Value (# %) / 1	Std. Uncertainty / Sum of Values
Absolute (Ave. Bias)	# %	Normal	1	Value (# %) / 1	Std. Uncertainty / Sum of Values
Subtotal of Standard Uncertainty Factors ($\sum u_i$)				Sum of values (above)	Sum of above (100 %)
Subtotal of the Sum of the Squares of the Uncertainty Factors ($\sum(u_i)^2$)				Sum of (Std. Uncertainty)^2	
Combined Standard Uncertainty U_c = square root of ($\sum(u_i)^2$)				SQRT(Subtotal (above))	
Expanded Combined Uncertainty (95% Confidence Level ; k=2) ; $U = (U_c * k)$				**= (Combined Std. Uncertainty)*2**	

5.1 Define Measurand and Process

The measurand is the quantity that is going to have its associated measurement uncertainty calculated and reported with that quantity. In essence, the measurand is the quantity being measured by an instrumental process within a procedure. The measurand will likely be determined by a combination of measurement processes within a procedure. If necessary, a reference to a specific standard operating procedure, instrument, instrumental technique, analytes, or group of analytes can be included within the measurement uncertainty documents. A list of dates where data is being pulled from should be on the measurement uncertainty spreadsheet. Names of the individual responsible for preparing and verifying the data will be present within the documents.

5.2 Evaluate and List Sources of Uncertainty

Quantitative measurements have sources of uncertainty that stem from items used within the process which can influence variations of the measurand data. Laboratory equipment such as weighing materials, volume-determining devices, reference materials, as well as human and envioronmental factors should be considered. Once components are identified as being significant to the measurement uncertainty process, they will be listed within the worksheet/spreadsheet. A component will be considered significant if removing the component will cause a change in the final expanded measurement uncertainty greater than the smallest numerical digit (e.g., 10 % to 11% or 0.005 grams to 0.006 grams).

Data from the specific measuring devices or analytical instrumention that are responsible for generating the measurand will be used in the calculations. Factors affecting measurement uncertainty may include, but not be limited to: types of samples (matrix, homogeneity), steps within sample preparation (extracting, diluting, concentrating), reference material uncertainties (Certificates of Analysis), measuring device uncertainties (balance/automatic pipette uncertainties), analytical instrumentation differences (systematic errors, random errors, precision, accuracy).

5.3 Quantify Sources of Uncertainty

Once the uncertainty components have been evaluated, the value of each will be determined. The approach to determining the uncertainty value is dependent on whether the uncertainty source is classified as a Type A or Type B. All uncertainties must be expressed in the same measurement unit. If the same measurement unit is not associated with each uncertainty, then each standard uncertainty can be converted into a percentage (i.e., relative standard uncertainty). The units for the uncertainty source values will be the same throughout the measurement uncertainty worksheet (e.g., % for Tox Unit and grams/milligrams for CS Unit).

5.3.1 Type A Uncertainty

Assuming a normal distribution, Type A uncertainty is evaluated by the statistical analysis of data from a series of measurements. Data from previous examinations of known reference standards or controls (e.g., positive control data) can establish relative standard deviation values for certain sources of uncertainty. Data from an uncertainty source can be listed within a number of events during a specified time period and the following statistical information is obtained: average, standard deviation, relative standard deviation, and number of samplings.

Average = sum of the number of measurments divided by the number of samplings
Standard Deviation = square root of the sum of the squared difference between measurement data points and the average of the data points, divided by one less than the number of measurement data points for a series of measurments.

$$\sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}}$$

Relative Standard Deviation = standard deviation divided by the average for a series of measurements.
Relative standard deviation values are also referred to as percent coefficient of variations (% CV)) within related measurements. If measurements are performed within a certain instrument using different parameters (e.g., chromatographic columns) for the same reference standard quantity being used as a control, then the largest %CV value should be used within the Type A Uncertainty field of the worksheet.

5.3.1.1 Measurements from the Controlled Sunstances (CS) Unit

Uncertainty measurements within the CS Unit will involve quantitations from a variety of different balances. Known certified reference weights will have had their masses recorded during quality assurance and quality control (QA/QC) practices by a variety of analysts during a specified time period. When measurement uncertainty calculations are to be conducted data from balances are used for Type A Uncertianty calculations. Multiple weights will be used when accumulating the data for each instrument and weights will be appropriate for each balance's measurement range.

5.3.1.2 Measurements from the Toxicology (Tox) Unit

Uncertainty measurements within the Tox Unit will involve quantitations from a variety of different analytes using different concentrations. Known certified reference drugs or metabolites are used for quality assurance purposes and their quantitative data are recorded. During a specified time period the data are used and measurement uncertainty calculations are calculated. The data from the analytical instrumentation (e.g., liquid chromatograph/mass spectrometers) will be used for Type A Uncertianty calculations. If an instrument has not been needed and used for quantitative determinations (e.g., gas chromatograph/mass spectrometer), then not enough data points will have been accumulated to tabulate an uncertainty measurement value for that technique and a standard 20% measurement uncertainty value will be used within reports.

Within recorded control data for QA/QC purposes, when positive control reference standard values fall out of a certain acceptable range (i.e., +/- 20% for drug, +/- 5% for volatiles) then data resulting from such analyses are not used in casework. Since they are not used in casework, corresponding control data are not used within measurement uncertainty calculations for related instrumental techniques.

5.3.2 Type B Uncertainty

When the statistical analysis of data from a series of observations is not used, then Type B uncertainty is evaluated. Examples of Type B sources of uncertainty include, but are not limited to: uncertainty values reported from an external calibration service, uncertainty values from a reference material's Certificate of Analysis (CoA), or values reported from literature regarding volumetric glassware. In these situations uncertainty values may be estimated using Rectangular Distribution or Triangular Distribution approaches. The Rectangular Distribution is the more conservative approach and should be used.

The approach to Rectangular Distribution can be used to estimate a *Type B* uncertainty component if the following criteria are met: the upper and lower limits of the device (a) are known, the probability that a value lies outside of these limits is zero, and one value is just as likely as another value between the limits (equal probability). The calculation to estimate the equivalent of one (1) standard deviation is defined as:

$$s = \frac{a}{\sqrt{3}}$$

5.4 Obtain Standard Uncertainty Values (u_i)

Standard uncertainty is obtained by dividing the uncertainty value by the distribution factor for each source of uncertainty. Measurement uncertainty values can be expressed as standard deviations. All statistically calculated uncertainty components (*Type A*, *Type B*- Rectangular Distribution) should already be expressed in one standard deviation (k-=1). To convert uncertainty values for each source of uncertainty into standard uncertainty values, the source values are divided by the divisor according to the type of distribution (i.e., normal, rectangular). When using Type B sources of uncertainty from areas such as calibration certificates or reference standard CoA documents, information must be carefully reviewed to determine what factors to use in order to obtain standard uncertainty figures. For certificates wherein k=2 (95% confidence level) and the distribution is rectangular, the reported uncertainty value on the certificate will need to be divided by the coverage factor (2* SQRT(3)) in order to arrive at the standard uncertainty (k=1).

5.5 Calculate combined standard uncertainty

The standard uncertainty values for each source are summed to get a subtotal of Standard Uncertainty Factors. Use this subtotal to obtain the percent each uncertainty source plays towards the standard uncertainty (aka. Relative Index). The sum of these percent factors must equal 100%.

Standard uncertainty values for each source of uncertainty is squared and then summed to get the Subtotal of the Sum of the Squares of the Uncertainty Factors. The square root of this value (along with appropriate units) is then taken and that result is called the Combined Standard Uncertainty for the method/measurand being evaluated.

5.6 Expand the combined standard uncertainty by coverage factor (k)

The value of the Combined Standard Uncertainty for the method/measurand being evaluated is based on a k-value of one (1). In order to have an appropriate k-value (e.g., 95.45% confidence interval (k=2) or 99.73% confifence interval (k=3), the Combined Standard Uncertainty value must be multiplied according to the desired coverage factor.

5.7 Express the expanded measurement uncertainty

Evaluate the final calculated expanded measurement uncertainty value for the techniques/analytes being measured to determine if it correlates with expected reporting values. If uncertainty numbers cause measurement results to be uninterpretable by customers, then a re-evaluation of the process must occur. Lead Examiners or higher must be consulted if resulting uncertainty values are expected to cause difficulty in interpreting quantitation results and may be unacceptable for use. Re-evaluation of the uncertainty calculations, review of data, or improvement of method may be necessary.

5.8 Report result with the associated uncertainty

Final measurment uncertainty values will be reported within all CAS reports wherein a quantitation is listed. Such values will have been rounded up so as to give the broadest range of values for the measurand being reported. Significant figure rules throughout the measurement uncertainty worksheet will not be followed and only during the final expanded uncertainty measurement will rounding-up occur. Measurement uncertainty reporting formats will follow appropriate accreditation guidelines, when applicable
(e.g., Diphenhydramine 60 ng/mL ± 19 ng/mL).

6. References

ANAB Policy on Measurement Uncertainty

ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty (Overview- AL-PD-3061, Annex A- AL-PD-3062, Annex B- AL-PD-3063, and Annex D- AL-PD-3065)

Joint Committee for Guides in Metrology (JCGM), *Evaluation of measurement data- Guide to the expression of uncertainty in measurement* (GUM) (GUM 1995 with minor corrections). (Sevres, France: International Bureau of Weights and Measures [BIPM]-JCGM 100, September 2008). Available at http://www.bipm.org/en/publications/guides/gum.html.

National Institute of Standards and Technology, *SOP 29 – Standard Operating Procedure for the Assignment of Uncertainty*, (Gaithersburg, Maryland, February 2012). Available at http://www.nist.gov/pml/wmd/labmetrology/upload/SOP_29_20120229.pdf.

Appendix:

Volatile Analyses by Headspace-Gas Chromatography (HS-GC/FID)		
Uncertainty for Ethanol		
Toxicology Unit		**06/01/2018 to 05/31/2019**
Items that influence method uncertainty (considered in determining uncertainty)		
Automatic Pipette/Diluter (Hamilton 500 Series) (Pipette #: MD91EE3472)	0.05 %	%CV value from calibrate company was 0.05% on 8/16/2018
Volumetric Pipet Uncertainty (Class A)	0.30 %	A 2 mL volumetric pipet was used to make 0.02% calibrator/control. The uncertainty was determined to be (accuracy volume/target volume)*100. The value is calculated to be 0.3%.
Volumetric Glassware (Class A)	0.08 %	Reported from Uncertainty of Volumetric Glassware printout (Fritz, J.S., Schenk, G.H., Quantitative Analytical Chemistry, 3rd ed., Allyl & Bacon, Boston, 1974, p.5) ; Only use volumetric flasks.
Balance Uncertainty - Mettler PG503-S	0.028 %	From 0.0014g / 5g ; Numerator is from the Certificate of Traceability (listed at 95% Confidence Level, k=2), denominator is from TX SOP because 5g of isopropanol is weighed (Date of balance calibration: 03/01/2018)
HS-GC/FID	1.486 %	Largest empirical Relative Standard Deviation (% CV) value selected from Positive Control (either CRM or In-House) Isopropanol Solutions (see attached table)
Absolute (Average Bias)	1.786 %	Largest average value indicating degree of accuracy. Obtained by replicate measurements of a reference material. [ABS(Empirical - Theoretical)) / (Theoretical)] x 100
Environmental Factors	Insignificant - This is demonstrated by daily instrument check and repeatability.	
Number of weighing events	Varies because Standard Stock and Volatile Stock solutions are made in monthly intervals.	
Sample loss during transfer	Not applicable with reference weights. Can be significant but is dependent on sample type. Minimized by users following good laboratory practice.	

Sources of Uncertainty	Value	Distribution	Divisor based on distribution	Standard Uncertainty (μ_i) (value/distribution)	Relative Index (% factor contributes to the std uncertainty) ($\mu_i/(\sum\mu_i)$)*100
Automatic Pipette/Diluter (Hamilton 500 Series) (Pipette #: MD91EE3472)	0.050 %	rectangular	√3	0.028867513	0.84
Volumetric Pipet Uncertainty (Class A)	0.300 %	rectangular	2*√3 ("2" needed for k=2 to be k=1)	0.08660254	2.52
Volumetric Glassware (Class A)	0.080 %	rectangular	√3	0.046188022	1.34
Balance Uncertainty - Mettler PG503-S	0.028 %	rectangular	2*√3 ("2" needed for k=2 to be k=1)	0.008082904	0.23
HS-GC/FID	1.486 %	Normal	1	1.486372907	43.18
Absolute (Average Bias)	1.786 %	Normal	1	1.785858586	51.88
Subtotal of Standard Uncertainty Factors ($\sum u_i$)	3.441972472	100.00			
Subtotal of the Sum of the Squares of the Uncertainty Factors ($\sum(u_i)^2$)				5.409	
Combined Standard Uncertainty U_c = square root of ($\sum(u_i)^2$)				2.326 %	
Expanded Combined Uncertainty (95% Confidence Level ; k=2) ; $U = (U_c * k)$				**5 percent**	

HS-GC(FID) Method-Isopropanol Uncertainty (CRM)					
		BAC 1	BAC 2	BAC 1	BAC 1
Instrument	DATE	CRM 0.1 g%	CRM 0.1 g%	Bias (%)	| Bias | (%)
HSGC-2	6/14/2018	0.1002	0.0993	0.20	0.20
HSGC-2	6/19/2018	0.0997	0.0992	-0.30	0.30
HSGC-2	6/26/2018	0.1009	0.1004	0.90	0.90
…	…	…	…	…	…
HSGC-2	6/24/2019	0.0975	0.0971	-2.50	2.50
HSGC-2	6/27/2019	0.0988	0.0988	-1.20	1.20
HSGC-2	6/27/2019	0.0985	0.0979	-1.50	1.50
	Ave	**0.0984**	**0.0980**	**1.558**	**1.786**
	Std. Dev.	**0.00130**	**0.00146**	**1.30**	**0.96**
	CV (%)	**1.3216**	**1.486**	**83.5**	**53.76**
Number of samples		**99**	**99**	**99**	**99**

Prepared by:		
Date:	**Printed Name**	**Signature**
Verified by:		
Date:	**Printed Name**	**Signature**

Note: Numbers within the spreadsheet have not been rounded, except for the final Expanded Combined Uncertainty value. What may appear as rounded numbers in the Uncertainty Budget are just for display purposes and may change depending on column width. The actual numbers within each cell are used for calculations.

Rev. #	History
1	New document.