

Title: Procedure 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 (with the exception of serum-ethanol conversions). This may be broken up by type of quantitative measurement and will be done annually. The required coverage probability of the expanded uncertainty will be a minimum of 95.45% (often referred to as '95%' or 'approximately 95%'). 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 procedures within the Chemical Analysis Section in which a quantitative measurement is reported (e.g., the quantity (mass/weight or volume) of evidence). Weights of drug residues 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.

3. Responsibilities

This document applies to Section personnel who report, or assist in the reporting of, measurement results within laboratory reports 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 simplified 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)

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	$\sqrt{3}$	Value (# %) / SQRT(3)	Std. Uncertainty / Sum of Values
Influence #2	# %	rectangular	$2*\sqrt{3}$ ("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
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. 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, analyte, 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(s) 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 environmental 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 instrumentation 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., grams or milligrams for Controlled Substances 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 measurements 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 measurements.

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

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.

Measurements from the Controlled Substances (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 Uncertainty calculations. Multiple weights will be used when accumulating the data for each instrument and weights will be appropriate for each balance's measurement range.

5.3.2 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 ($2 * \text{SQRT}(3)$) 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% confidence 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 the method may be necessary (e.g., Ethanol 0.025 g% \pm 0.030 g%).

5.8 Report result with the associated uncertainty

Final measurement uncertainty values will be reported within all CAS reports wherein a quantitation is listed (with the exception of serum-ethanol conversions). 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., Weight 0.048 grams \pm 0.007 grams).

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.

CAS-04 - Measurement Uncertainty

Document ID: 14886

Revision: 4

Effective Date: 11/3/2022

Status: Published

Page 9 of 11

Approved by Director: Dr. Guy Vallaro

Appendix (Examples):

Date: 07/13/2022	Mettler top loading MS16001L (#13) (Serial No.: B112123015)
Specifications per manufacturer documentation	
Weighing Range:	16.2kg

Items that influence balance uncertainty (considered in determining uncertainty)

Readability	0.1g	From Model Specification Sheet - the smallest increment of displayed measurement.
Repeatability	0.0562g	Largest empirical std. deviation value selected from daily weight values and any additional weighings (see attached table)
Linearity	0.2g	From Model Specification and Certificate of Traceability sheets - whichever value is larger will be listed.
Uncertainty (formerly "Accuracy")	0.18g	Reported from Certificate of Calibration (listed at 95% Conf. Level ; k=2). Used largest value within reported range on certificate.
Environmental Factors	Insignificant - This is demonstrated by daily instrument check and repeatability	
Number of weighing events	Varies	
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 (u_i) (value/distribution)	Relative Index (% factor contributes to the std. uncertainty) ($u_i/(\sum u_i) * 100$)
Readability	0.1g	rectangular	$\sqrt{3}$	0.057735027	20.52
Repeatability	0.1g	normal	1	0.056232156	19.98
Linearity	0.2g	rectangular	$\sqrt{3}$	0.115470054	41.03
Measurement Unc (formerly "Accuracy")	0.18g	rectangular	$2*\sqrt{3}$ ("2" needed for k=2 to be k=1)	0.051961524	18.47
Subtotal of Standard Uncertainty Factors ($\sum u_i$)				0.281398761	100.00
Subtotal of the Sum of the Squares of the Uncertainty Factors ($\sum (u_i)^2$)				0.02252872	
Combined Uncertainty U_c = square root of ($\sum (u_i)^2$)				0.1501 g	
Expanded Combined Uncertainty U (95% Confid. Level ; k=2) ; U = ($U_c * k$)				0.4 grams/weighing event	
Previous Expanded Combined Uncertainty U (95% ; k=2) in 2021				0.4 grams per weighing event	

CAS-04 - Measurement Uncertainty*Approved by Director: Dr. Guy Vallaro*

Document ID: 14886

Revision: 4

Effective Date: 11/3/2022

Status: Published

Page 10 of 11

Balance #13	Date Range: 05/01/2021 to 05/23/2022 ; Balance Uncertainty Limits (< 16.2kg)							
	Date	20g	Date	100g	Date	500g	Date	1000g
1	3/3/2022	20.0	5/11/2021	100.0	6/9/2021	500.0	6/30/2021	1000.1
2	5/4/2022	20.0	5/13/2021	100.1	6/28/2021	500.0	9/30/2021	1000.0
3	5/4/2022	20.0	6/9/2021	100.0	9/20/2021	500.0	10/14/2021	1000.0
4	5/4/2022	20.0	7/27/2021	99.8	10/13/2021	500.1	1/10/2022	1000.0
5	5/4/2022	19.9	8/30/2021	100.0	10/18/2021	500.0	5/4/2022	1000.0
...
20	5/4/2022	20.0	5/3/2022	100.0	-	-	5/4/2022	1000.0
21	5/4/2022	20.0	5/4/2022	100.0	-	-	5/4/2022	1000.0
22	-	-	5/12/2022	100.0	-	-	5/4/2022	1000.0
23	-	-	5/17/2022	100.0	-	-	5/4/2022	1000.1
24	-	-	-	-	-	-	5/4/2022	1000.0
Average (g)		19.9714 3		99.9957		500.0111		999.9958
Std. Dev. (g)		0.05606		0.056232156		0.0333333		0.0550033
% Rel. Std. Dev.		0.28071		0.056235		0.006667		0.005500

The following individuals have reviewed the numbers and verify that the calculations are correct:

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
2	Added the serum-ethanol conversion exception within Section 2 and Section 5.8. General formatting changes throughout document. Removal of 'Bias' rows in tables from examples in Section 5 and within the Appendix. Reworded Section 5.3.1.2 to include either previous uncertainty values or a standard 20% value for instances where low data are present. Added an example in Section 5.7. Changed 'Isopropanol' to 'Ethanol' within the Appendix.
3	Updated Section 2 to include the frequency of updating uncertainty budgets and other information. Updated the format within the 'History' section.
4	Removed information regarding toxicology from Sections 2, 5.3.1.2, 5.3, and 5.8. Updated appendix to include removing toxicology-related information. Minor formatting and grammatical updates were made throughout the document.