

A. PURPOSE:

Determination of Uncertainty of Measure is a method of defining and quantifying the magnitude of the parameters associated with a process that may contribute to the error, or uncertainty inherent in that process. Since all measurement has a potential for variability, determination of the uncertainty associated with a process, allows users of such measurements to understand the reliability and hence suitability of the measured values for the intended use.

The Controlled Substance laboratory reports the uncertainty for most cases where a weight criteria may be approached or exceeded or when drug purities are determined. For reporting weights, uncertainty of measure is considered for cases where the substance identified has a criteria weight (either State or Federal) associated with it. This is true for whether the weight is determined by simple weight determination using an analytical balance or for weight determinations using quantitation of the analyte in question. For information on criteria weights see SOP CS-5.1 Uncertainty Action Levels. This SOP describes the methods used to determine or develop the Uncertainty budgets for the CS laboratory.

B. RESPONSIBILITY:

All individual assigned to developing uncertainty budgets within the CS section are required to follow the guidelines set forth in this SOP.

C. DEFINITIONS:

1. Uncertainty of Measure⁴ is a parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurand.
2. Measurement that Matters⁴: A determined value that is used, or may reasonably be used, by an immediate or extended customer (anyone in the Judicial process) to determine, prosecute, or defend the type or level of criminal charge(s).
3. Type B Evaluation²: method of evaluation of uncertainty by means other than the statistical analysis of a series of observations
4. Readability: the smallest increment which the balance displays (i.e 0.01g or 0.001g)

5. Repeatability: closeness of the agreement between the results of successive measurements of the same item carried out under the same conditions (example: a balances ability to consistently deliver the same weight for a given mass)
6. Linearity: the quality of delivering a significantly identical sensitivity throughout the weighing capacity of a balance
7. Standard Uncertainty² (u_i): a component of uncertainty, represented by an estimated standard deviation equal to the positive square root of the estimated variance.
8. Distribution:
 - a. Normal²: A pattern of frequency of values arrayed around a central mean value, such that the pattern is consistent with a Gaussian distribution
 - b. Rectangular²: A distribution of values that that there is equal probability that a value lies anywhere within the interval.
9. Combined Standard Uncertainty² (u_c): square root of the sum of the squares of the uncertainty factors, used to express the uncertainty of many measurement results.
10. Coverage Value (k): when applied to the combined uncertainty allows for the definition of the confidence interval; ($k = 2$ allows for a 95% confidence interval, $k = 3$ allows for a 99% confidence interval)
11. Expanded Uncertainty¹ (U): the interval in which a value (y) can be confidently asserted to lie
12. Index: demonstrates the individual factor's contribution to the event uncertainty
13. Standard Deviation: A value associated with a normal, or Gaussian distribution describing an average departure from the mean value.

D. PROCEDURE:

When uncertainty is determined, the traceability of the steps involved must be established. Tools used to assure measurement traceability include:

1. Use of ISO 17025 vendors for calibration services.
2. Review the scope of accreditation for calibration services to assure their scope includes the needs of the laboratory.
3. Use of ISO accredited vendors as the source of Certified Reference materials.
 - a. Review of the COA (Certificate of Analysis) to determine the "true" concentration with reported uncertainty range.

4. Assessment of the capability of the vendor to provide the supply or calibration service needed when an ISO accredited vendor cannot be used. Documentation of this review will be maintained with the Quality Section.

Equipment having significant effect on results where uncertainty is reported will be calibrated by a calibration laboratory; according to the following schedule.

1. Masses: annually
2. Balance: annually – earlier if the balance is moved or there are other indications that the balance requires maintenance such as drift.
3. Pipettes: annually earlier if indicated by improper function. In-house periodic checks may be performed following SOP CS-XXX if determined to be needed by the section supervisor.
4. Class A glassware – will be replaced if visual defects are observed; no periodic calibration is required.

Note: in general annual calibrations will occur +/-30 days of the anniversary date.

E. Developing an Uncertainty Budget Associated for Weighing

(For an example of an uncertainty budget spread sheet see below, an Excel spreadsheet may be used to perform all the needed calculations).

1. Specify the measurement process (i.e. identify the measurand)
2. Determine what aspects of the procedure can influence the value obtained:
 - a. Instrument Readability –obtained from manufacturer’s instrument manual, most balances will have two ranges and the Uncertainty should be calculated for both ranges.
 - b. Instrument Linearity - obtained from manufacturers instrument manual
 - c. Instrument Repeatability – this may be given in the manufactures specifications for the instrument however for the purposes of these budgets repeatability will be determined in the laboratory by one of the following methods:
 - i. Use data obtained during the daily use of the instrument; obtain the values obtained from the daily instrument check, to determine the standard deviation. This is acceptable to use when there are sufficient data points for masses over the working range of the instrument (a minimum of ten data points at the low range, middle and high range of

the range is desired). Use worksheet SOP CS-2.1 to compile the data and calculate the standard deviation.

- ii. Weigh a certified mass multiple times (minimum twenty) then calculate the standard deviation for the readings. It is preferred to use masses throughout the range of the instrument (or normal use of the instrument) and use the largest standard deviation in the calculation.
 - (a) Example for a balance that has a range of 0.001g to 300g, weigh masses at 200 mg, 100g and 300g if available. See worksheet SOP CS-2.1
 - iii. It is also acceptable to use a combination of historical data and data obtained specifically for the purpose of determining the uncertainty budget to calculate the standard deviations.
 - d. Environmental conditions – although it is important to understand that these items may have some influence on uncertainty, these generally will not significantly affect the work performed in the CS laboratory and are not therefore, included in the uncertainty budget.
 - i. Temperature
 - ii. Humidity
 - iii. Air flow
 - e. Sample loss during weighing
3. Determine to what extent the factor affects the overall uncertainty budget. An item that contributes less than 1% to the budget could be eliminated from the budget.
 4. Calculate the standard uncertainty based on the type of distribution the data represents ($\sum(u_i)^2$)
 - a. Calculate the Index: Determine if the item represents 1% or more of the total uncertainty, if it does not it does not need to be included in the budget.
 5. Calculate the Combined Standard Uncertainty: U_c = square root of ($\sum(u_i)^2$)
 6. Calculate the Expanded Combined Uncertainty using the desired coverage factor.
 - a. Coverage factors: 2 will give a 95% Confidence Interval, 3 will provide a 99% Confidence Interval – The CS laboratory will calculate based on a coverage factor of 2.

7. Evaluate the expanded uncertainty.
 - a. Review for accuracy of calculations made
 - b. Does the expanded uncertainty make logical sense?
 - i. Evaluate to determine if it meets the needs of the customer. An uncertainty that is overly large may not provide helpful information to the end user of the report. An expanded uncertainty of +/-50% could not give practical information to the client.
8. This calculated value will be used and reported as a Confidence Interval as appropriate (see SOP CS-5).

Example of budget chart for weights:

Low Range: (<110g)				
Factor	Value (x), g	Uncertainty of the individual factors (u_i), g	Distribution	Index (Relative contribution to u_i in %)
Readability	From manufacture	$x/\text{distribution value}$	Rectangular (use the square root of 3 as the distribution value)	The uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_i)^2 / (\sum(u_i)^2)$
Repeatability	Determined in house this is the SD determined as listed above	Since this is a normal distribution the value is the SD obtained from the calculations	Normal – (normal distributions needs no estimation of the value since it has been calculated)	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_i)^2 / (\sum(u_i)^2)$
Linearity	From manufacture	$x/\text{distribution value}$	Rectangular (use the square root of 3 as the distribution value)	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_i)^2 / (\sum(u_i)^2)$
Subtotal of the uncertainty ($\sum(u_i)^2$)		Sum of the square of each of the uncertainty factors		
$U_c = \text{square root of } (\sum(u_i)^2)$	Square root of the sum of the squared uncertainty components	grams		
Expanded Uncertainty (U); where (k) = 2	$U_c \times \text{the coverage factor}$ $U = (u_c \times 2)$	gram/weighing event		

F. Developing an Uncertainty Budget for Drug Purity Determination:

In the Controlled Substance laboratory purity may be performed when requested, for possible medical diversion cases or possible unusual concentration of illicit drugs are encountered.

Uncertainty will be determined separately for liquid versus solid materials. The overall uncertainty budget determination will address the same concepts but each type of quantitation will include the method specific devices.

The uncertainty determination/budget is maintained in a laboratory notebook within the Controlled Substance section.

1. Specify the measurement process (i.e. what is the measurand)
2. Determine what aspects of the procedure can influence the value obtained. Quantitation requires the uncertainty of several devices and/or pieces of laboratory equipment; the uncertainty of each portion is added to determine the combined uncertainty. Items to evaluate may include:
 - a. Liquid Matrix:
 - i. Uncertainty of Pipette uses for preparation for controls, calibrators and samples.
 - (a) Repeatability (historical data)
 - (b) Accuracy (From annual calibration certificate or manufacturer's specification)
 - (c) Analyst variation (historical control data –if available)
 - (d) Environmental variation (historical data)
 - ii. Balance uncertainty – when powdered standards are used.
 - (a) Readability (From manufacturer's specifications)
 - (b) Repeatability (Historical data)
 - (c) Linearity (from manufacturer's specifications)
 - (d) Environmental variation (historical data)
 - (e) Analyst variation (historical data)
 - (i) Note: the uncertainty (not expanded uncertainty) developed per scale can be used for this step.
 - (f) Uncertainty of volumetric glassware used.

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- (g) Accuracy (per manufacturer's specification)
- (h) Analyst variation (historical data – if available)
- (i) Environmental Conditions
- iii. Value of Standards/Controls per the COA – with the related uncertainty.
- b. Solid Dose Matrix:
 - i. Uncertainty of Pipette uses for preparation for controls, calibrators and samples.
 - (a) Repeatability (historical data)
 - (b) Accuracy (From annual calibration certificate or manufacturer's specification)
 - (c) Analyst variation (historical control data –if available)
 - (d) Environmental variation (historical data)
 - ii. Balance uncertainty – when powdered standards are used.
 - (a) Readability (From manufacturer's specifications)
 - (b) Repeatability (Historical data)
 - (c) Linearity (from manufacturer's specifications)
 - (d) Environmental variation (historical data)
 - (e) Analyst variation (historical data)
 - (i) Note: the uncertainty (not expanded uncertainty) developed per scale can be used for this step.
 - iii. Uncertainty of volumetric glassware used.
 - (a) Accuracy (per manufacturer's specification)
 - (b) Analyst variation (historical data – if available)
 - (c) Environmental Conditions
 - iv. Value of Standards/Controls per the COA – with the related uncertainty.
- 3. Quantify the uncertainty components.
 - a. Each component is evaluated to determine if they can be factored into the total uncertainty, and to what extent they contribute to the total uncertainty.
 - b. Components that contribute less than 1% to the total uncertainty need not be included in the calculation of the expanded uncertainty.

- c. Documentation of this evaluation is maintained in the Uncertainty Notebook within the Controlled Substance section.
4. Calculate the standard uncertainty based on the distribution type of the data evaluation ($\sum(u_i)^2$).
 - a. If the distribution type is determined to be Normal the value is divided by 1
 - b. If the distribution type is determined to be Rectangular the value is divided by the square root of 3.
5. Calculate the combined standard uncertainty, U_c = square root of ($\sum(u_i)^2$).
6. Calculate the Expanded Combined Uncertainty using the desired coverage factor.
 - a. Coverage factors: 2 will give a 95% Confidence Interval, 3 will provide a 99% Confidence Interval – The CS laboratory calculates the coverage factor based on a coverage factor of 2.
7. Evaluate the expanded uncertainty.
 - a. Review for accuracy of calculations made
 - b. Does the expanded uncertainty make logical sense?
 - i. Evaluate to determine if it meets the needs of the customer. An uncertainty that is overly large may not provide helpful information to the end user of the report. An expanded uncertainty of +/-50% could not give practical information to the client.
8. This calculated value is used and reported as a Confidence Interval.
 - a. Uncertainty is reported in the same units as the obtained quantitated value. The units must be S.I units.
 - b. The confidence interval is defined in the report.
 - i. Example for a liquid quantitation: X mg/ml +/-Y mg/ml; confidence interval = 95%
 - ii. Example for a solid dose quantitation: X% +/-Y%; confidence interval = 95%

*Approved by Director: Dr. Guy Vallaro***G. DOCUMENTATION:**

Documentation for the calculation of uncertainty budgets is maintained within the Controlled Substance section in the Uncertainty notebook.

H. SOURCES OF ERROR:

1. Not considering all substantial contributors in the uncertainty budget
2. Applying the wrong type of distribution based on the data

I. REFERENCES:

¹SWGDRUG Supplemental Document SD-3 “Quality Assurance/Uncertainty
www.swgdrug.org

²“NIST Reference on Constants, Units and Uncertainty”
<http://physics.gov/cuc/uncertainty>

³General Metrological Terms: <http://iso.org>

⁴ASCLD/LAB International “Estimating Uncertainty of Measurement Policy”

*Approved by Director: Dr. Guy Vallaro***Example of Uncertainty Budget Calculation:****Denver Instrument Company Top Loading Balance TR-603D #5**

Specifications per Manufacturer

Weighing

Range 610/110g

Readability 0.01/0.001g

Linearity 0.01/0.002g

Repeatability 0.01/0.001g

Items that influence balance uncertainty (considered in determining uncertainty)Readability⁽¹⁾ 0.01/0.001g per manufactures specifications and current balance validation reportRepeatability⁽²⁾ based on daily instrument check using certified massesLinearity⁽³⁾ 0.01/0.002g per manufacturers specificationsAccuracy⁽⁴⁾ 0.02g per annual balance certification

Environmental Factors insignificant, demonstrated by daily instrument check, repeatability

Number of weighing events will vary per event

Sample loss during transfer Can be significant, based on the nature of the samples weighed in the section. Procedures attempt to minimize this.

Low Range: (<110g)

Sources of Uncertainty	Value (grams)	Distribution	Divisor based on distribution	Standard Uncertainty (ui) (value/distribution)	Relative Index (% factor contributes to the standard uncertainty) (ui/($\sum ui$))*100
Readability ⁽¹⁾	0.001	rectangular	$\sqrt{3}$	0.00057735	2.52
Repeatability ⁽²⁾	0.0039	normal	1	0.0039	16.99
Linearity ⁽³⁾	0.002	rectangular	$\sqrt{3}$	0.001154701	5.03
Accuracy	0.02	rectangular	$2*\sqrt{3}$ (to reduce k=2 to 1)	0.017320508	75.46
Subtotal of Standard Uncertainty factors ($\sum ui$)				0.022952559	
Subtotal of the sum of the squares of the uncertainty factors ($\sum (u_i)^2$)				0.00031688	
Combined Uncertainty Uc = square root of ($\sum (u_i)^2$)				0.01780103	grams
Expanded Combined Uncertainty U where k = 2				0.03560206	grams/weighing event