A. **PURPOSE**:

Determination of the Uncertainty of Measurements 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 Analysis Unit (CSAU) reports uncertainty for most cases were a weight criteria may be approached or exceeded or when drug purities are determined. For reporting weights, uncertainty of measure is considered for cases were 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 Analysis Unit.

~~The Controlled Substance section need only report uncertainty when the reported weight (without packaging) brackets a criteria weight by 100 times the highest uncertainty for the balance type. See SOP CS 5.1 for guidance.~~

The Controlled Substance Analysis Unit will report uncertainty values when exact weights, volumes, or other quantitative numbers are listed in a report. Approximate values will not have an accompanying uncertainty value in reports. See SOP CS 5.1 for guidance.

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 Measure [4] 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 Matters [4]: 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 Evaluation [2]: 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 Uncertainty [2] (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. Normal [2]: A pattern of frequency of values arrayed around a central mean value, such that the pattern is consistent with a Gaussian distribution
   b. Rectangular [2]**:** A distribution of values that that there is equal probability that a value lies anywhere within the interval.
9. Combined Standard Uncertainty [2]**:** (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 Uncertainty [1] **(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 ensure measurement traceability include:

1. Use of ISO 17025 vendors for calibration services.
2. Review of the scope of accreditation for calibration services to ensure 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 Certificate of Analysis (COA) 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 than scheduled if the balance is moved and there are indications that the balance requires maintenance (e.g., drift in weight measurement).
3. Pipettes: annually (earlier than scheduled if pipettes are not functioning properly. ~~In-house periodic checks may be performed following SOP CS-XXX if determined to be needed by the section supervisor.~~
4. Class A glassware: No periodic calibration is required. Glassware will be replaced when necessary (e.g., significant visual defects are observed).

   Note: Annual calibrations should occur within +/-30 days of the date since the last calibration occurred.

E. Developing an Uncertainty Budget Associated for Weighing

(For an example of an uncertainty budget spreadsheet 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. Use the values obtained from the daily instrument check to determine the standard deviation. These values are 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 region, middle region, and high region 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.

Example: For a balance that has a range of 0.001g to 300g, weigh masses at 200mg, 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 in order to calculate the standard deviations.

d. Environmental conditions – although it is important to understand that environmental factors 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~~. However, these environmental factors will be incorporated during normal weighing procedures that are encountered within step c.

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($\sum(u_i)^2$)

   Calculate the Index: Determine if the item represents 1% or more of the total uncertainty. If it not, then it doesn't need to be included in the budget.

5. Calculate the Combined Standard Uncertainty: **Uc** = square root of ($\sum(u_i)^2$)
6. Calculate the Expanded Combined Uncertainty using the desired coverage factor.

   Coverage Factors: Two factors will give a 95% Confidence Interval, three factors will provide a 99% Confidence Interval – The CS Analysis Unit 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/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/distribution value	**Rectangular** (use the square	The standard uncertainty for the factor divided by

			root of 3 as the distribution value)	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		
Uc = square root of $(\sum(u_i)^2)$	Square root of the sum of the squared uncertainty components	grams		
Expanded Uncertainty (U); where (k) = 2	Uc*the coverage factor U =(u_cx2)	**gram/weighing event**		

F. Developing an Uncertainty Budget for Drug Purity Determination:

~~In the Controlled Substance laboratory~~ Purity (i.e., quantitative analyses) may be performed when specifically requested or when needed with certain types of cases (e.g., medical diversion, adulteration, unusual concentrations of illicit drugs are encountered).

~~Connecticut laws base drug charges on the aggregate weight of a substance with no consideration of the purity. Therefore the information is for informational use only and not one that will influence an imposed penalty. Based on this information uncertainty need not be always be reported. The section Supervisor or Deputy Director should be consulted in cases where quantitation is requested.~~

~~Currently the laboratory upon specific request performed quantitation/determination of purity for the following types of cases:~~

1. ~~Overdose cases where the submitting agency is trying to determine if they have a drug of high concentration being distributed.~~
2. ~~Diversion cases where the submitting agency is trying to determine if a health practitioner is stealing or diluting drugs for personal use.~~
3. ~~Federal Cases were the submitting agency is trying to prove a specific amount of pure drug. These cases will require uncertainty be reported.~~

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 CSA Unit. Listed below is the general approach to calculating uncertainty for both liquid and solid substances.

1. Specify the measurement process (i.e., what is the measurand)

2. Determine what factors of the quantitative procedure significantly influence the final value. Uncertainty involves the quantitative variability of several devices and/or pieces of laboratory equipment. The uncertainties from equipment used within a quantitation is gathered and a combined uncertainty is calculated. Items to evaluate may include:
    a. Liquid Matrix:
        i. Uncertainty of pipettes used 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, if available)
        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) (Note: the uncertainty (not expanded uncertainty) developed per scale can be used for this step)
            (f) Uncertainty of volumetric glassware used.
            (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, **Uc** = 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 Analysis Unit 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%

G. Steps to consider for Calculating Uncertainty for Drug Quantitation cases:

Solids:

Step	Uncertainty Component to Consider
X grams is weighed	Balance
X mL of solvent is added	Pipette or Volumetric Flask (if serial dilutions are required this is considered for each dilution)
X µL is added to X µL of internal standard	Pipette (both used or if the same used twice the one x2)
	Uncertainty of the standard used to prepare the calibrator, this will be on the certificate of analysis.*

Liquids:

Step	Uncertainty Component to Consider
X µL of liquid measured	pipette
Above added to X volume of liquid	Pipette or Volumetric flask (if serial dilutions are required this is considered for each dilution)
X µL added to X µL of internal	Pipette (both used or if the same used

standard	twice the one x2)
	Uncertainty of the standard used to prepare the calibrator, this will be on the certificate of analysis.*

***** The controls need not be added since they are an independent step. They are used to assess the acceptability of the run but do not influence the result.

H. **DOCUMENTATION**:

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

I. **SOURCES OF ERROR**:

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

J. **REFERENCES**:

[1]SWGDRUG Supplemental Document SD-3 "Quality Assurance/Uncertainty www.swgdrug.org

[2]"NIST Reference on Constants, Units and Uncertainty" http://physics.gov/cuc/uncertainty

[3]General Metrological Terms: http://iso.org

[4]ASCLD/LAB International "Estimating Uncertainty of Measurement Policy'

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 report
Repeatability(2)	based on daily instrument check using certified masses
Linearity(3)	0.01/0.002g per manufacturers specifications
Accuracy(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/(∑ui))*100
Readability (1)	0.001	rectangular	√3	0.00057735	2.52
Repeatability (2)	0.0039	normal	1	0.0039	16.99
Linearity (3)	0.002	rectangular	√3	0.001154701	5.03
Accuracy	0.02	rectangular	2*√3 (to reduce k=2 to 1)	0.017320508	75.46
Subtotal of Standard Uncertainty factors (∑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 ($\sum$(ui)2)				0.01780103	grams
Expanded Combined Uncertainty U where k = 2				**0.03560206**	**grams/weighing event**