การประเมินค่าความไม่แน่นอนของการวัด ในงานวิเคราะห์ยา

The Estimation of Measurement Uncertainty in Pharmaceutical Anaylsis

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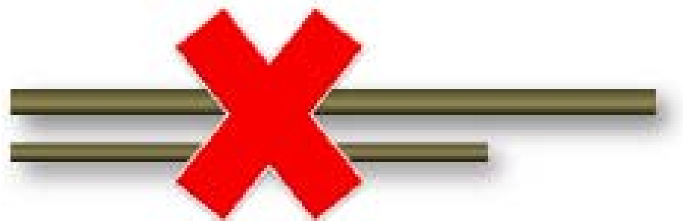
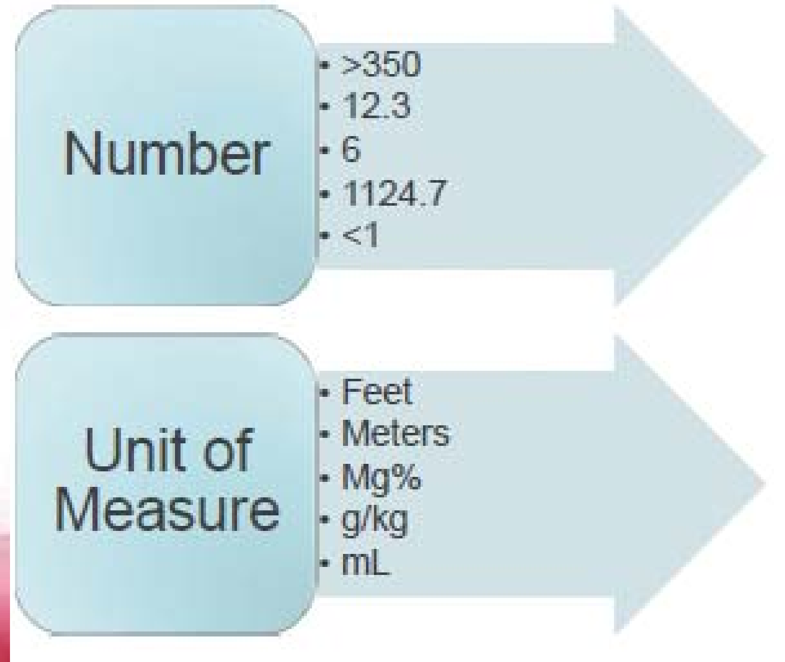
What is not a measurement?

- There are some processes that might seem to be measurements, but are not.
  - Counting is not normally viewed as a measurement, it is a number.
  - Comparing two pieces of string to see which is longer is not really a measurement.
- A measurement usually has two components, a number and a unit of measure.





What is a measurement?

Measurement is:

- A set of operations that determine the value of a quantity.
- It tells us about a property of something (how heavy, hot, or long an object is).
- They are made using an instrument (e.g., rulers, weighing scales, stopwatches, thermometers, etc.)
- A measurement is taken by comparing an unknown quantity, such as the length of the stick we looked at previously… with a standard unit like a ruler.
- The instruments used to make these comparisons can be simple ones, like rulers, scales, stopwatches and thermometers or they can be complex scientific instruments.

Logic of a measurement

- A **True value** is obtained only by a perfect measurement.
- No measurement system is perfect!
- Therefore, we can never obtain a **true value.**
- So. some **statistical predicatability** must be provided in a measurement.

What is measurement uncertainty (MU)?

- MU is the doubt that exists about the result of any measurement.
- You might think that well-made rulers, thermometers, and scales always give the right answer. But for every measurement - even the most careful one - there is always a margin of doubt.
- This is expressed in our everyday lives as 'give or take'.

My cat weighs twelve pounds, give or take an ounce or two.

What is Uncertainty?

Uncertainty : A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand.

(ISO 17025 Definition)

: A parameter associated with the result of a measurement that defines the range of values that could reasonably be attributed to the measured quantity.

(UKAS Definition)

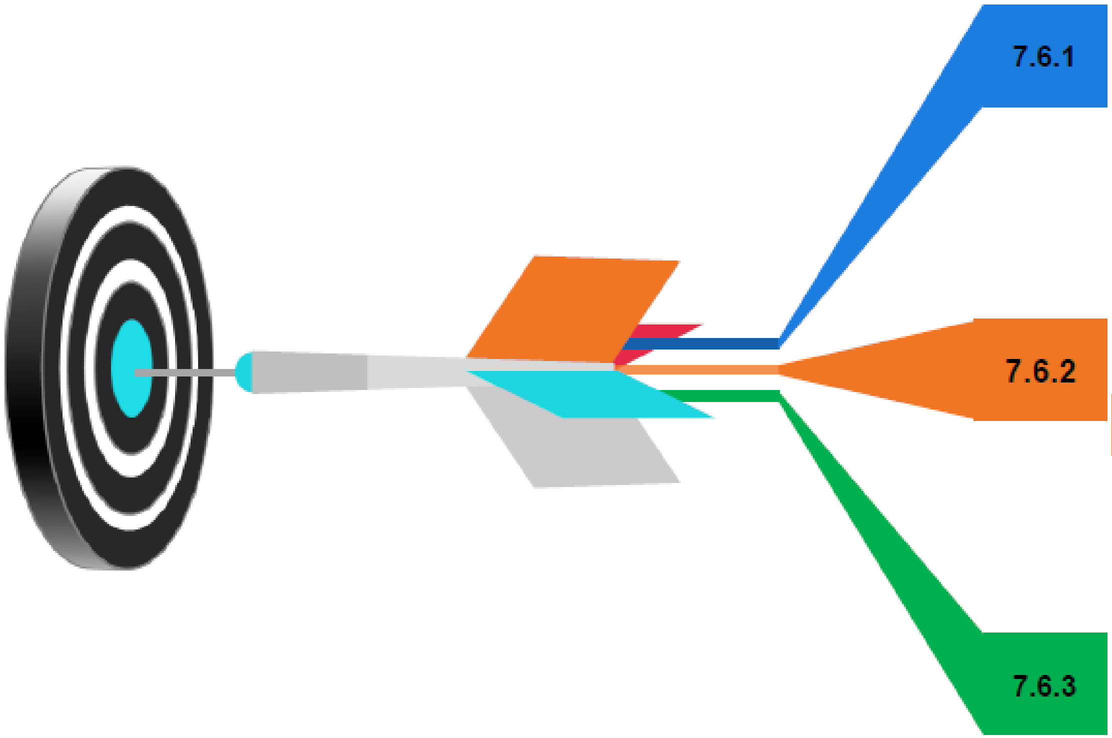
Why is it important?

1. It gives an answer to the question, how well does the result represent the value of the quantity being measured?

2. The standard ISO/IEC 17025:2017 [*General requirements for the competence of testing and calibration laboratories*] specifies requirements for reporting and evaluating uncertainty of measurement. (Section 7.6)

Measurement Uncertainty in ISO/IEC 17025:2017

7.6 Evaluation of measurement uncertainty



Laboratories shall identify the contributions to measurement uncertainty

A laboratory performing calibrations, including of its own equipment, shall evaluate the measurement uncertainty for all calibrations

A laboratory performing testing shall evaluate measurement uncertainty

Guidelines for Estimation of Measurement Uncertainty

ISO/IEC Guide 98-3 : Guide to the expression of uncertainty in measurement

EURACHEM/CITAC : Guide Quantifying Uncertainty in Analytical Measurement (2012)

UKAS : The expression of uncertainty and confidence in measurement (2012)

NIST : Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results (1994)

Terminology in Measurement Uncertainty

Measurand : **Particular quantity subject to measurement.**

Measurement : **Set of operations having the object of determining a value of a quantity.**

Uncertainty : **Parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand.**

Traceablity : **The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties**

Terminology in Measurement Uncertainty

Standard Uncertainty: u (x_i)

Uncertainty of the result x_i of a measurement expressed as a standard deviation

Combined standard uncertainty: uc (y)

Standard uncertainty of the result y of a measurement when the result is obtained from the values of a number of other quantities, equal to the positive square root of a sum of terms, the terms being the variances or covariances of these other quantities weighted according to how the measurement result varies with these quantities.

Terminology in Measurement Uncertainty

***Expanded uncertainty* :** **U(x)**

Quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand.

***Coverage factor* :** **k**

Numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty.

Sources of uncertainty in chemical analysis

Physical measurement

- Weighing
- Temperature measurement
- Volume measurement

Chemical measurement

- Bias
- Precision

The concept of evaluation of measurement uncertainty

Results

↓

Sources of uncertainty

↓

Standard uncertainty $u(x_i)$ | **Standard uncertainty $u(x_i)$** | **Standard uncertainty $u(x_i)$**

↓

Combined standard uncertainty $u_c(y)$

↓

Expanded uncertainty $U(x)$

General Approach of Measurement Uncertainty

Step 1
Description of the measurement procedure

→ Flow chart with the description of all steps of the analytical procedure

Step 2
Specification of the measurand

→ Relationship between the measurand and variables of the analytical procedure

Step 3
Identification of uncertainty sources

→ The uncertainty of each variable is identified by mean of a cause and effect diagram

Step 4 & 5
Quantification and combination of uncertainty

→ The above identified uncertainties are quantified and combined to give the total uncertainty on the measurand

Step by Step

for evaluating
and calculating the uncertainty measurement

Determination of Potency of Penicillin V Potassium by HPLC

Step 1

Description of the measurement procedure

Summarise the tasks that need to be performed in order to obtain an estimate of the uncertainty associated with a measurement result.

(SOP, WI, Lab results, method validation data, etc.)

Example

Determination of Potency of Penicillin V Potassium by HPLC

Summary of Method

- **Prepare Standard Solution** - Accurately weigh 25 mg. of Pen. V Pot. RS of stated purity in to a 10 ml. volumetric flask. and dilute to volume with mobile phase.
- **Prepare Sample Solution** - Accurately weigh 125 mg. of Pen. V Pot. Sample in to a 50 ml. volumetric flask. Dilute to volume with mobile phase.
- **Measure Standard Solution** - Inject a volume of standard solution. Record peak area.
- **Measure Sample Solution** - Inject a volume of sample solution. Record peak area.
- **Determine potency of Pen. V Pot. in sample**

$$Potency(unit/mg) = \frac{A_{sam} \times V_{sam} \times C_{std} \times P_{std}}{A_{std} \times M_{sam}}$$

Step 2
Specification of the measurand

This step requires :

- A clear and unambiguous statement of what is being measurand.
- A quantitative expression relating the value of the measurand to the parameters on which it depends.

Measurand = concentration of an analyte

1. Specify measurand

Measurand = Potency of Pen. V Pot. (unit/mg)

$$Potency(unit/mg) = \frac{A_{sam} \times V_{sam} \times C_{std} \times P_{std}}{A_{std} \times M_{sam}}$$

Asam	= Peak area for the sample solution
A std	= Peak area for the standard solution
C std	= Concentration of the standard solution (mg/ml)
M sam	= Mass of sample (mg)
v sam	= Volume of sample solution (ml)
P std	= Potency of standard = 1520 unit/mg

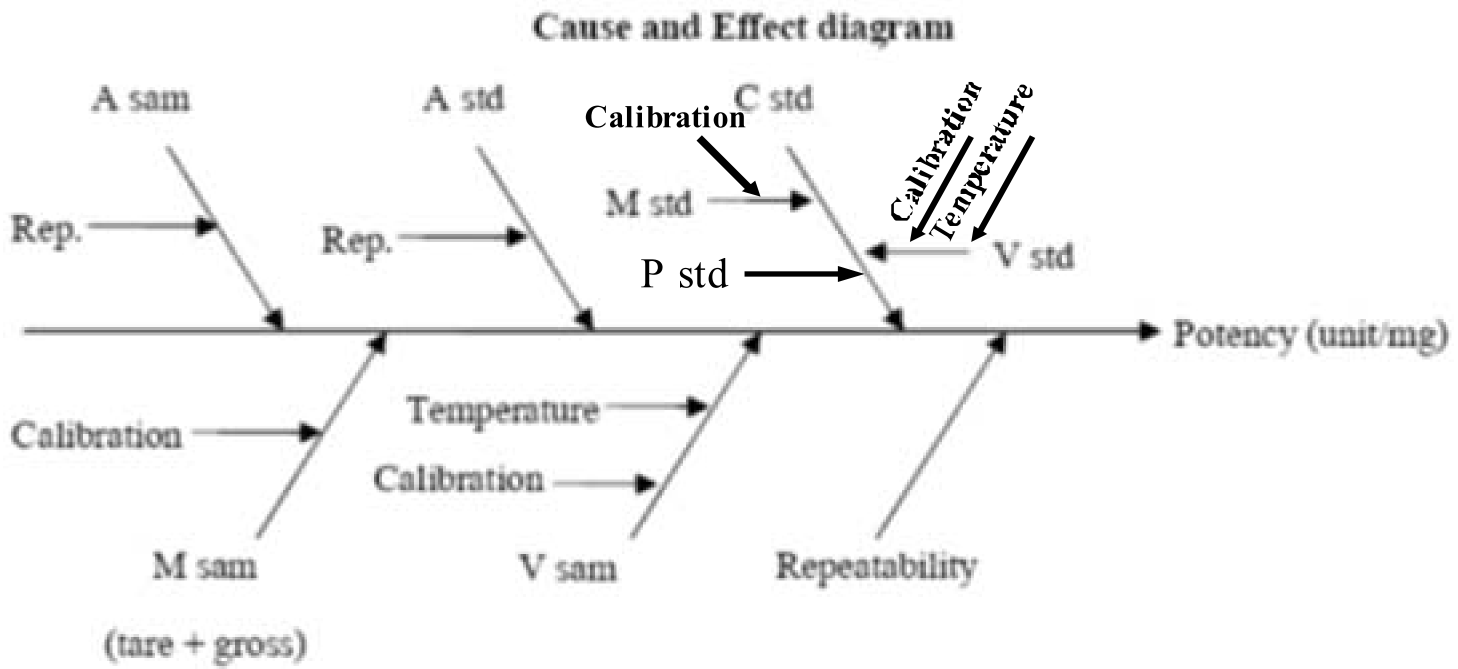
DATA

	Standard	Sample A	Sample B	Sample C	Sample D
Tare+Load (g)	0.038566	0.2413	0.2411	0.2417	0.2429
Tare (g)	0.013645	0.1162	0.1162	0.1162	0.116
Wt. Taken (g)	0.024921	0.1251	0.1249	0.1255	0.1269
Injection	Peak area	Peak area	Peak area	Peak area	Peak area
1	1955253	1928275	1925840	1923644	1945017
2	1957281	1936523	1925759	1916104	1951982
3	1957966	1930579	1920420	1916049	1954756
4	1955178				
5	1953689				
6	1956137				
Mean	1955917.3	1931792.3	1924006.3	1918599.0	1950585.0
SD	1552.9519	4255.7619	3106.119819	4369.1847	5017.542925
%RSD	0.08	0.22	0.16	0.23	0.26
Potency (unit/mg)		1495.3116	1491.6695	1480.3658	1488.4417
Mean		1488.95			
SD		6.372121176			
%RSD		0.428			

Av. 1931245.65

Step 3
Identification of uncertainty sources

List the possible sources of uncertainty on cause and effect diagram



Step 4
Quantification of uncertainty

Classification of components of uncertainty

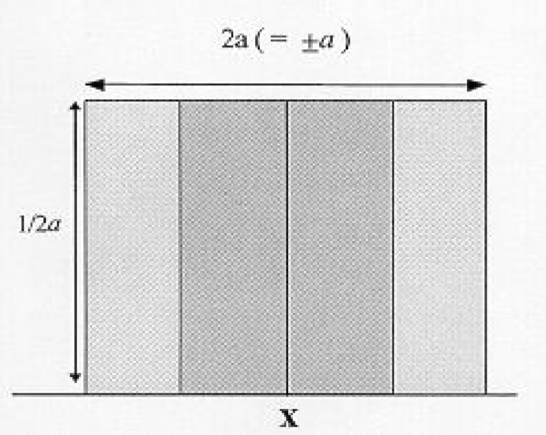
1. **Type A** : Sources which are evaluated by statistical method (Repeatability, repeated observation)
2. **Type B** : Sources which are evaluated by other means (Calibration certification data, factor of environment)

Classification of data distributions

Rectangular distribution
Triangular distribution
Normal distribution

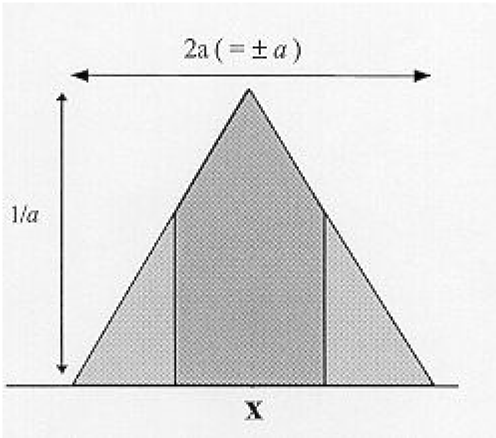
Classification of data distributions

Rectangular Distribution

Form	Use when:	Uncertainty
	-A certificate or other specification gives limits without specifying a level of confidence (e.g. 25ml ± 0.05ml) -An estimate is made in the form of a maximum range (±) with no knowledge of the shape of the distribution.	$u(x) = \frac{a}{\sqrt{3}}$

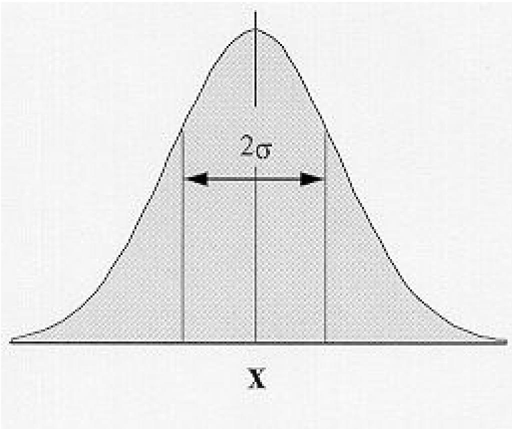
Classification of data distributions

Triangular Distribution

Form	Use when:	Uncertainty
	-The available information concerning *x* is less limited than for a rectangular distribution. Values close to *x* are more likely than near the bounds. -An estimate is made in the form of a maximum range (±) described by a symmetric distribution.	$u(x) = \frac{a}{\sqrt{6}}$

Classification of data distributions

Normal Distribution

Form	Use when:	Uncertainty
	- An estimate is made from repeated observations of a randomly varying process.	$u(x) = s$
	- An uncertainty is made from a calibration certificate give limits that specifying a level of confidence.	$u(x) = U/k$
	-An uncertainty is made from manufacturers' specifications that specifying a level of confidence.	$u(x) = \frac{\text{Tolerance limit}}{k}$

Combination of standard uncertainties

Two simple rules for combining standard uncertainties are given :

Rule 1 **: For model (equation) involving only a sum or difference of quantities (y = p+q+r+…), the combined standard uncertainty $U_c(y)$ is given by**

$$u_c(y(p,q\ldots)) = \sqrt{u(p)^2 + u(q)^2 + \ldots}$$

Rule 2 **: For model (equation) involving only a product of quotient (y = pxqxr…), (y = p/(qxr) the combined standard uncertainty Uc(y) is given by**

$$u_c(y) = y \cdot \sqrt{\left(\frac{u(p)}{\bar{p}}\right)^2 + \left(\frac{u(q)}{\bar{q}}\right)^2 + \ldots}$$

Step 4
Quantification of uncertainty

Source of Uncertainty	Quantify Procedure
1. Uncertainty of A sam.	Repeatability of sample: Standard deviation of peak area of sample solution
2. Uncertainty of A std.	Repeatability of standard: Standard deviation of peak area of standard solution
3. Uncertainty of M sam.	Max. Uncertainty of Balance from Calibration Certificate
4. Uncertainty of V sam.	- Tolerance of volumetric flask - Temperature Effect
5. Uncertainty of C std. 5.1 Wt. of Std. 5.2 Vol. of Std. Sol.	 - Max. Uncertainty of Balance from Calibration Certificate - Tolerance of volumetric flask **P std = 0** - Temperature Effect
6. Uncertainty of Method Precision	Repeatability of sample: Relative standard deviation of potency of sample

Quantifying uncertainty from Purity of Standard (P std)

พิจารณาจาก Certificate of Analysis ดังนี้

1. กรณีไม่ระบุค่า uncertainty

   1.1 มีค่า purity เป็น 100% เช่น USPRS, BPCRS เป็นต้น ไม่ต้องพิจารณาค่า u

   1.2 ระบุ specification เป็น range ให้หาค่ากลาง แล้วลบด้วย 100 จากนั้นนำค่าที่ได้แปลงค่าเป็น standard uncertainty (u)

   เช่น 96-100% ค่ากลางเท่ากับ 98 นำ 100-98= 2 , u $= 2/\sqrt{3}$

   1.3 ระบุค่าต่ำสุด ให้นำค่าต่ำสุดลบด้วย 100 จากนั้นนำค่าที่ได้แปลงค่าเป็น standard uncertainty (u)

   เช่น มีความบริสุทธิ์ อย่างน้อย 98% คำนวณ 100-98 = 2 , u $= 2/\sqrt{3}$

2. กรณีระบุค่า uncertainty ให้แปลงค่านั้นเป็น standard uncertainty (u)

   เช่น ความบริสุทธิ์ 99.66±0.54 : นำ $0.54/\sqrt{3}$

3. กรณี ไม่พบข้อมูลใดๆ ให้ถือว่าความไม่แน่นอนมีค่าน้อยมากจนตัดทิ้งได้

Standard Uncertainty

1. Peak area of Sample

Maximum standard deviation of peak area of sample solution

Standard deviation = 5017.543

Standard Uncertainty of A sam , $u(A_{sam}) = \pm 5017.543$

2. Peak area of Standard

Standard deviation of peak area of standard solution

Standard deviation = 1552.9519

Standard Uncertainty of A std , $u(A_{std}) = \pm 1552.9519$

3. Mass of Sample (tare + gross)

Max. Uncertainty of Balance from Calibration Certificate = ± 0.00039 g., k = 2.25

Standard Uncertainty = (± 0.00039/2.25) = ± 0.00017 g. = ± 0.17 mg.

Contributed by twice (tare + Gross)

$$u(M_{sam}) = \sqrt{0.17^2 + 0.17^2} = 0.2404$$

Standard Uncertainty of M sam , $u(M_{sam}) = \pm 0.24$ mg

4. Volume of Sample

- Calibration

Tolerance of volumetric flask = ± 0.06 ml,

Standard uncertainty is calculated as a Rectangular distribution (divisor = √3)

Standard Uncertainty (u_{CAL}) = ± 0.06/√3 = ± 0.0346 ml

- Temperature Effect

The coefficient of volume expansion for water = 2.1 x 10^{-4} $°C^{-1}$

Laboratory temperature varies between 3 °C

Volume variation = ± (50 x 3 x 2.1 x 10^{-4}) = ± 0.0315 ml.

Standard uncertainty is calculated as a rectangular distribution (divisor = √3)

Standard Uncertainty (u_{TEMP}) = ± 0.0315/√3 = ± **0.0182 ml.**

Combining these contributions:

$$u(V_{sam}) = \sqrt{u_{CAL}{}^2 + u_{TEMP}{}^2}$$

$$= \sqrt{0.0346^2 + \mathbf{0.0182^2}}$$

$$= \mathbf{0.0391}$$

Standard uncertainty of V sam , u(V_{sam}) = ± 0.0391 ml

5. Concentration of Standard Solution

5.1 Volume of standard solution

- Calibration

Tolerance of volumetric flask = $\pm$ 0.04 ml,

Standard uncertainty is calculated as a Rectangular distribution (divisor = $\sqrt{3}$)

Standard Uncertainty (u_{CAL}) = $\pm$ 0.04/$\sqrt{3}$ = $\pm$0.0231 ml

- Temperature Effect

The coefficient of volume expansion for water = 2.1×10^{-4} °C^{-1}

Laboratory temperature varies between _ 3 °C

Volume variation = $\pm(10 \times 3 \times 2.1 \times 10^{-4}) = \pm 0.0063$ ml.

Standard uncertainty is calculated as a rectangular distribution (divisor = $\sqrt{3}$)

Standard Uncertainty (u_{TEMP}) = $\pm$ 0.0063/$\sqrt{3}$ = $\pm$0.0036 ml

Combining these contributions:

$$u_V = \sqrt{u_{CAL}{}^2 + u_{TEMP}{}^2}$$
$$= \sqrt{0.0231^2 + 0.0036^2}$$
$$= 0.0234$$

Standard uncertainty of V std., u_V = $\pm$0.0234 ml

5.2 Mass of Standard (tare + gross)

Max. Uncertainty of Balance from Calibration Certificate = ±0.026 mg., k = 2.05

Standard Uncertainty = (±0.026/2.05) = ±0.0127 mg.

Contributed by twice (tare + Gross)

$$u(M) = \sqrt{0.0127^2 + 0.0127^2} = 0.01796$$

Standard uncertainty of M std. , u_M = ±0.01796 mg

Standard uncertainty of concentration of standard solution

$$C = \frac{M}{V}$$

$$u(c_{std}) = c_{std}\sqrt{\left(\frac{u_v}{v}\right)^2 + \left(\frac{u_M}{M}\right)^2}$$

$$= 2.4921\sqrt{\left(\frac{0.0234}{10.0}\right)^2 + \left(\frac{0.01796}{24.921}\right)^2}$$

$$= 0.00610$$

Standard uncertainty of C std , $u(C_{std})$ = ±0.00610 mg/ml

6. Repeatability

Assay: 4 replications, mean = 1488.95 unit/mg, SD = 6.37, RSD = 0.00428

Standard Uncertainty = relative standard deviation

= ±0.00428/

Standard uncertainty due to Repeatability , $u_{(Rep.)}$ = ±(0.00428

Step 5
Combination of uncertainty

	Source	value	Unit	Std. Uncert.	Rel. Std. Uncert.
1	A std	1955917.3	-	**1552.952**	0.00079
2	A sam	**1931245.65**	-	5017.543	0.00257
3	M sam	**125.6**	mg	0.24	0.00189
4	V sam	50.0	ml	**0.0391**	**0.00078**
5	C std	2.4921	mg/ml	0.00610	0.00245
6	Repeatability	1.0	unit/mg	**0.00428**	**0.00428**

$$Potency(unit/mg) = \frac{A_{sam} \times V_{sam} \times C_{std} \times P_{std}}{A_{std} \times M_{sam}}$$

$$= \frac{1931245.65 \times 50.0 \times 2.4921 \times 1520}{1955917.3 \times 125.6}$$

= 1488.95 unit /mg

$$\frac{u_c(Potency)}{Potency} = \sqrt{\left(\frac{u(A_{sam})}{A_{sam}}\right)^2 + \left(\frac{u(A_{std})}{A_{std}}\right)^2 + \left(\frac{u(M_{sam})}{M_{sam}}\right)^2 + \left(\frac{u(V_{sam})}{V_{sam}}\right)^2 + \left(\frac{u(C_{std})}{C_{std}}\right)^2 + u(prec.)^2}$$

$$= \sqrt{0.00257^2 + 0.00079^2 + 0.00189^2 + 0.00078^2 + 0.00245^2 + 0.00428^2}$$

$$= 0.005977$$

$$u_c(Potency) = Potency \times 0.005977$$

$$= 1488.95 \times 0.005977$$

$$= 8.90$$

Combined standard uncertainty = ± 8.90 unit/mg

Step 5
Combination of uncertainty

Expanded uncertainty (U)

The final stage is to multiply the combined standard uncertainty by the chosen coverage factor(k) in order to obtain an expanded uncertainty

In the testing lab, it is recommended that k is set to 2 for 95% confidence level

Step 5
Combination of uncertainty

Reporting uncertainty

The result X should be stated together with the expanded uncertainty U

Result : X ± U (units)

Example

Total nitrogen : 3.52 ± 0.14%w/w

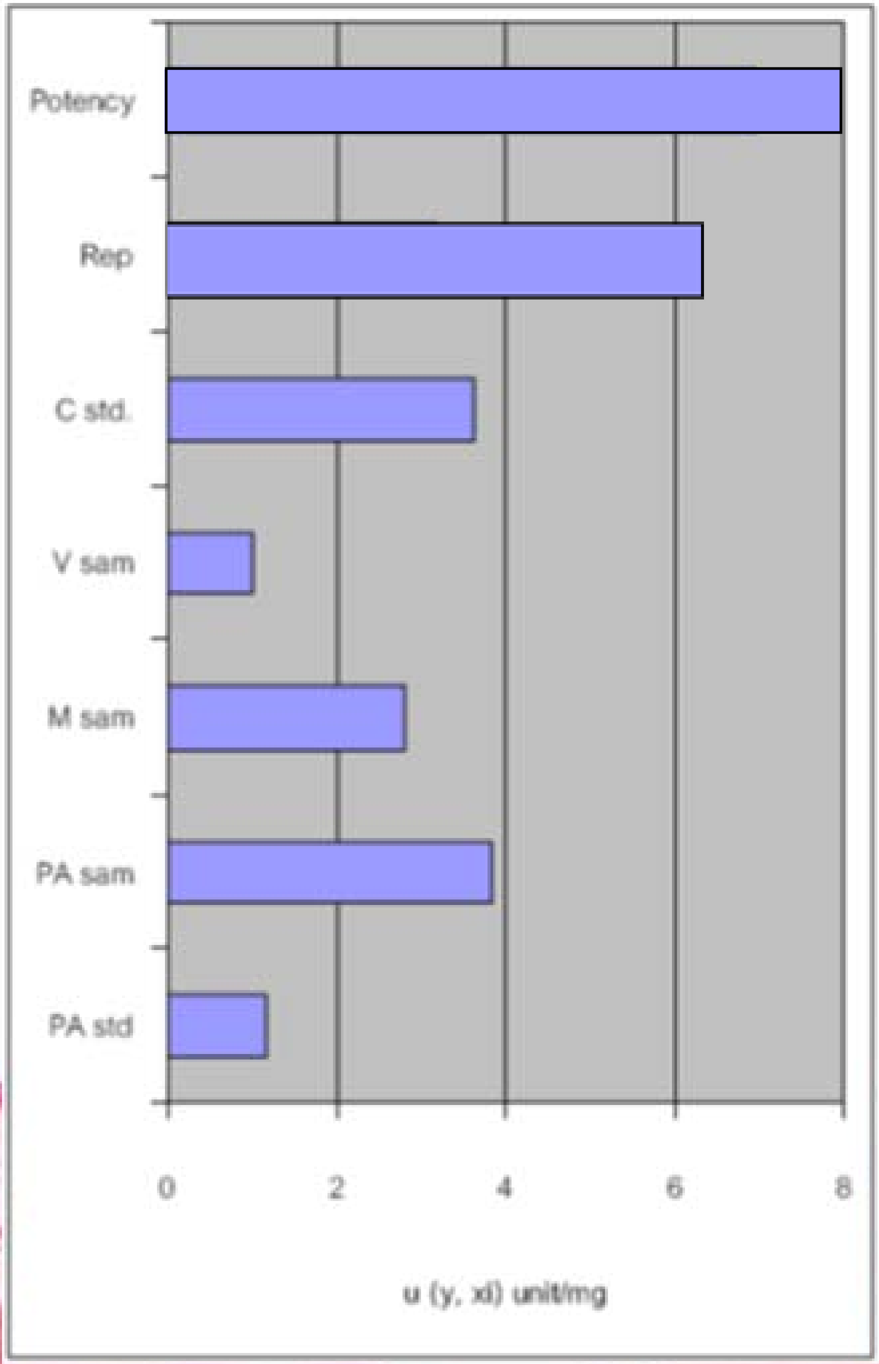
The report uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%

Expanded Uncertainty

The expanded uncertainty, U(Potency$_{Pen\ V}$) is calculated by multiplying the combined standard uncertainty by a coverage factor of 2:

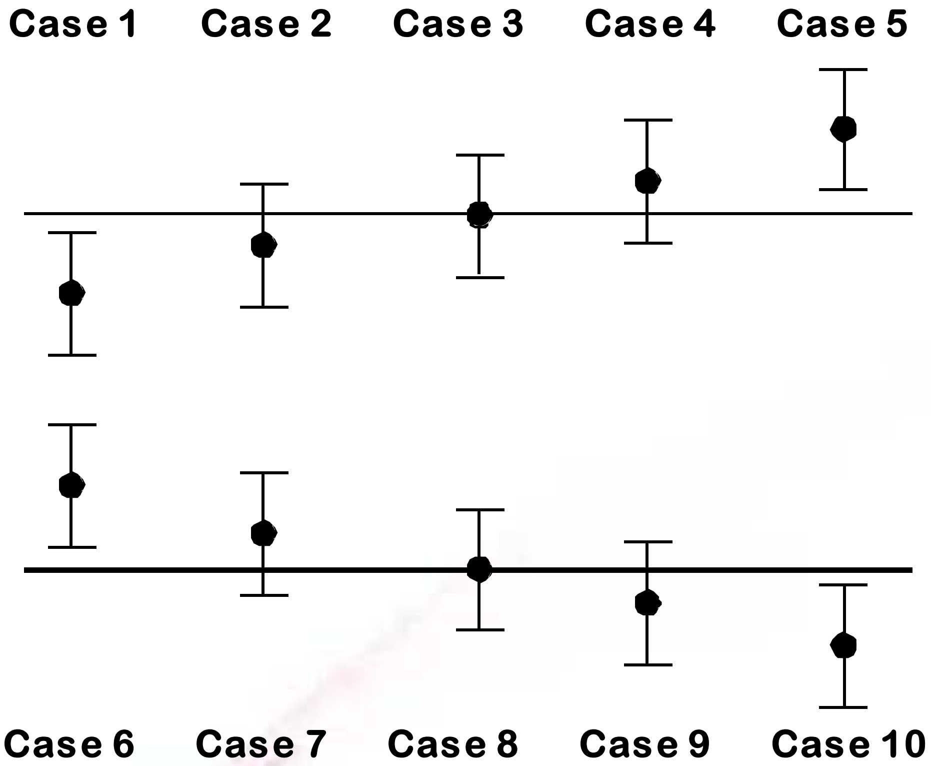
U(Potency$_{Pen\ V}$) = ±8.90 x 2 = ± 17.80 unit/mg

The potency of the Pen. V is 1488.95 ± 17.80 unit/mg



Potency
Rep
C std.
V sam
M sam
PA sam
PA std
0
2
4
6
8
u (y, xi) unit/mg

Uncertainty and Compliance limits



Summary

1. The measurement uncertainty is a quantitative indication of the quality of measurement and result.
2. The measurement uncertainty is a parameter for comparison of results from different laboratories.
3. The uncertainty contribution can be used for consideration of improvement of method in the future.
4. The measurement uncertainty is required for accredited laboratories in ISO/IEC 17025:2005

“ Maturity of the mind is the capacity to endure uncertainty ”

John Finley (1908-1974) English Historian and Mathematician

Thank you