SADCWater PT Chemistry workshop 2018 – Part 3: **Uncertainty of Measurement**

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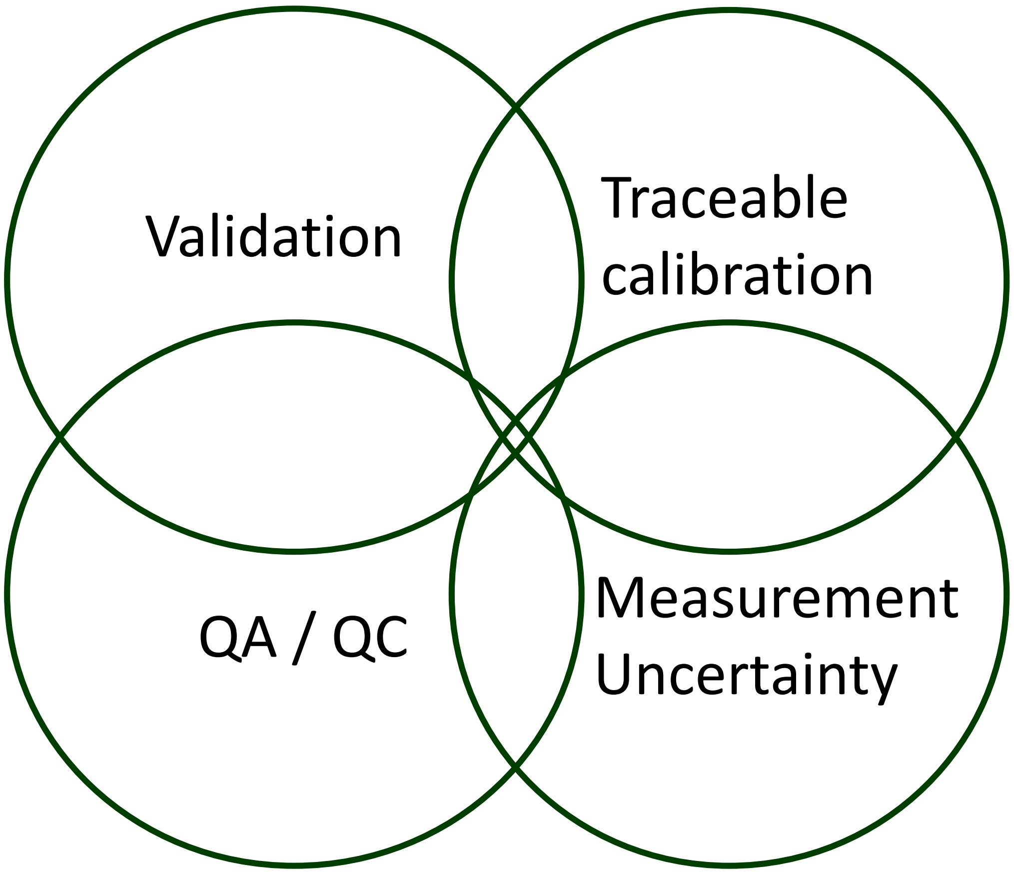
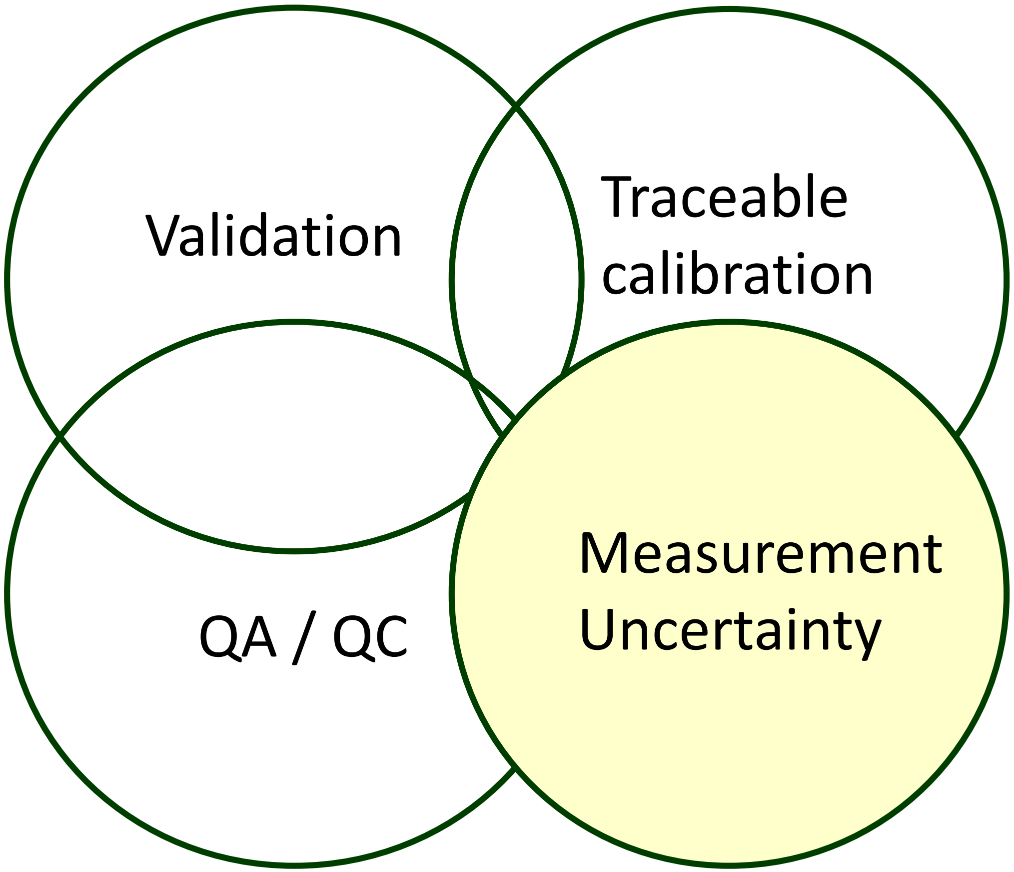
26-27 November 2018

Ensuring valid Analytical Measurements



Ensuring valid Analytical Measurements



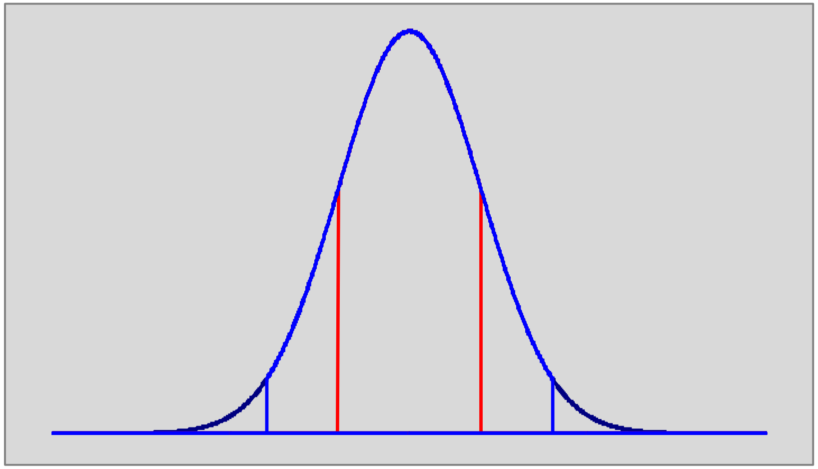
Overview

- What is uncertainty of measurement (UoM)?
- Different approaches to evaluate UoM
  - Bottom Up
  - Top Down
- Basic steps
  - Top Down (simplified)
  - Bottom Up
- Expanded uncertainty

Normal Distribution

Important Properties

- Approximately 68% (68,27%) of the data lie within μ ± 1σ
- Approximately 95 % (95,45%) of the data lie within μ ± 2σ
- Approximately 99,7 % (99,73%) of the data lie within μ ± 3σ

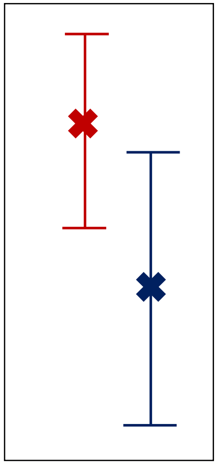


Measurement Uncertainty vs S_{Rw}

- Within laboratory reproducibility: S_{Rw}
  - Possible variation in analytical results if same sample submitted to the laboratory at different times
- Measurement uncertainty
  - Possible maximum deviation of a single result from the mean or reference value, if the same sample is submitted to several competent laboratories

What is uncertainty of measurement?

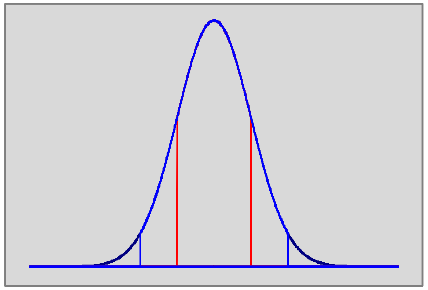
- It tells us something about how much you can trust the measurement i.e. the quality of the measurement
- We need two numbers to quantify uncertainty:
  - The width of the margin of doubt, the confidence interval, and
  - The confidence level, how sure we are that the true value is within the margin of doubt.



$$m = 1000.00250 \pm 0.00050\ g\ *$$

** At level of confidence of 95%*

UoM: Basic concepts



- Standard Uncertainty

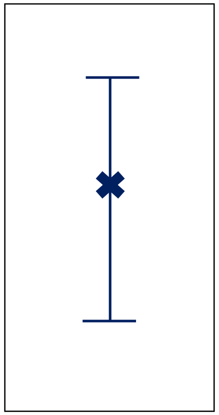
$$u(x_i) @ LOC = 68\%$$

- Combined Standard Uncertainty

$$u_c(y) = \sqrt{\sum (c_i \cdot u(x_i))^2} \quad \text{OR} \quad u_c(y) = \sqrt{\sum \left(\frac{u(x_i)}{x_i}\right)^2}$$

- Expanded Uncertainty

$$U = k \times u_c(y)$$



k = Coverage factor associated with:

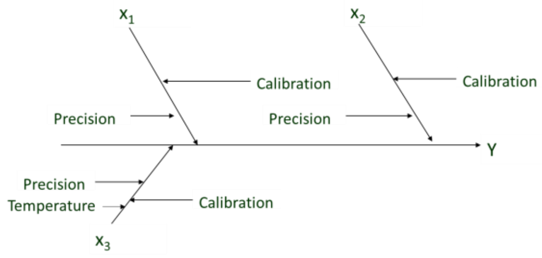
- Level of Confidence (LOC)
- Degrees of Freedom

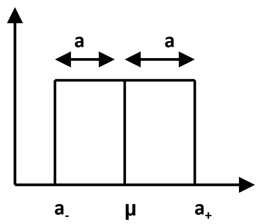
Evaluation: UoM

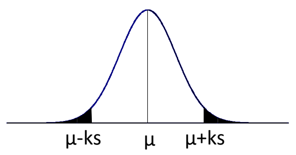
- Several approaches:
  - Bottom - Up:
    - Based on mathematical model describing the complete measurement procedure
    - Critical to identify all parameters during modelling
    - GUM
  - Top - Down:
    - Use of method validation and quality control data to estimate the uncertainty of measurement
    - No knowledge of model required
    - Approaches: EURACHEM / CITAC, NORDTEST

UoM: Basic steps

- Identify the uncertainty sources
  - Equation / Fishbone
- Quantify individual uncertainties
  - Type A & B uncertainties, OR
  - Reproducibility & Bias
- Calculate combined uncertainty
  - GUM, EURACHEM, NORDTEST
- Calculate the expanded uncertainty
  - $U = k \times u_c$



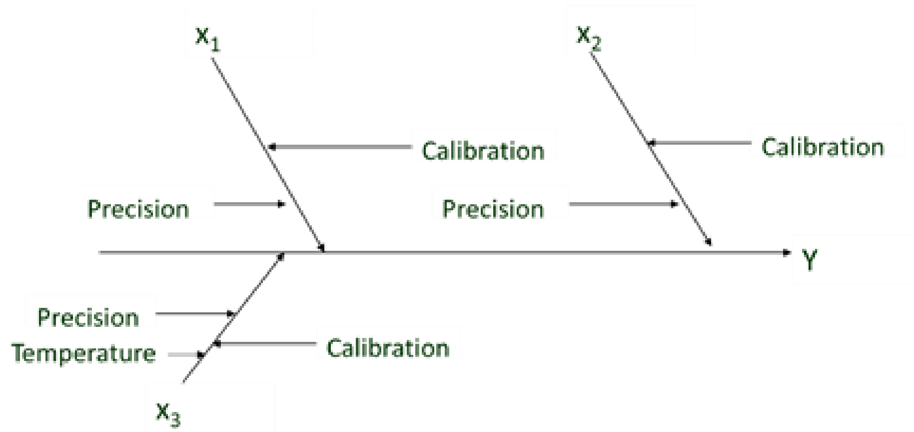




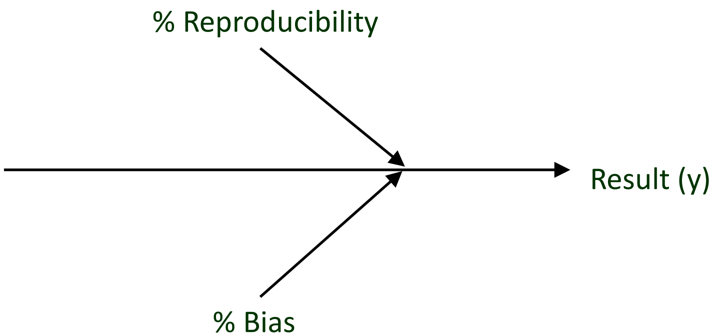
Identify uncertainty sources

- Mathematical Model
  - Bottom-Up

$$Y = \frac{x_1 \times x_2}{x_3}$$



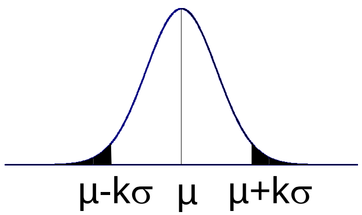
- Method Validation data
  - Top-Down



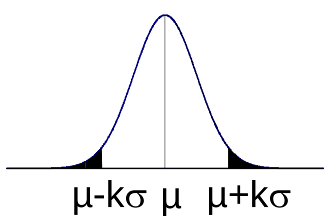
- Bottom Up Approach

Quantify individual uncertainties

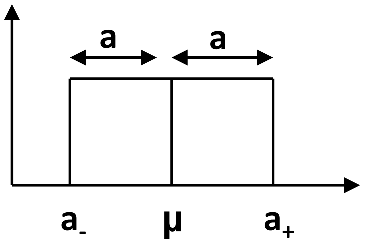
- **Type A:** Estimate and associated uncertainty are directly determined by the current measurement (Statistics)
  - Normal distribution:



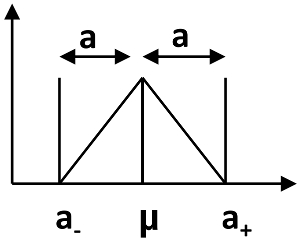
- **Type B:** Estimate and associated uncertainty are brought into the measurement from external sources (Other sources)
  - Normal distribution



Rectangular distribution

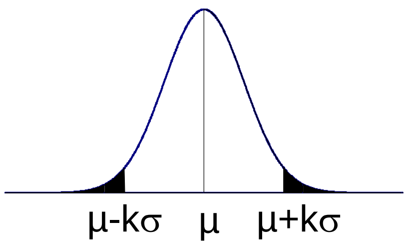


Triangular distribution



Quantify individual uncertainties

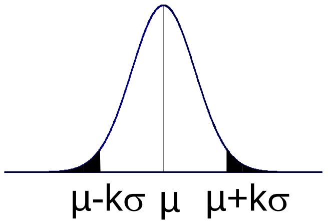
- Type A:
  - For component of uncertainty arising from random effects
  - Applied when multiple independent observations are made under the same (REPEATABILITY) conditions
  - Normal (Gaussian) probability distribution
  - Examples:
    - Sample analysed in repeatedly:
      - Same day, same analyst, same instrument



$$u(x) = \frac{s(x)}{\sqrt{n}}$$

Quantify individual uncertainties

- Type B:
  - For component of uncertainty arising from random effects
  - Applied when results originate from REPRODUCIBILTY conditions
  - Normal (Gaussian) probability distribution
  - Examples:
    - Results from Certificate of Analysis e.g. CRM
      - Level of confidence / Expanded Uncertainty
    - Method validation data from e.g. control charts
      - Standard deviation



$$u(x) = \frac{U(x)}{k}$$

$$u(x) = s(x)$$

Type B: Normal Distribution

- **Example:**
- Standard uncertainty (As concentration) associated with a certified calibration standard solution:

Number	QC1488-20ML
Lot	019373
Solvent (Matrix)	Water, 5% Nitric
Hazard	Toxic, Corrosive
Storage &Handling	Store at room temperature.
Expiration Date	See Sample Label
Certification Date:	11/2/2012
Certified By:	Christopher Rucinski - QA Director

Analyte		*Units*	*Certified Value*[1,4]	***k*** [5]
Arsenic, As 1010	Traceable to: NIST SRM 3103a Lot 010713	µg/L	19.4 ± 0.423	1.96
Beryllium, Be 1020	Traceable to: NIST SRM 3105a	µg/L	2.71 ± 0.0614	1.96
Cadmium, Cd 1030	Traceable to: NIST SRM 3108 Lot 060531	µg/L	13.7 ± 0.155	1.96

Type B: Normal Distribution

- **Example:**
- Standard uncertainty (As concentration) associated with a certified calibration standard solution:

- Value:

  Conc (As) = 19.4 µg/L

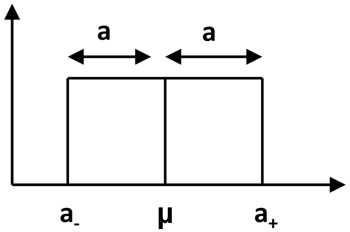
- Standard Uncertainty:

$$u(x) = \frac{U(x)}{k}$$

$$u(x) = \frac{0{,}423}{2.00} = 0.216\ \mu g/L$$

Quantify individual uncertainties

- Type B:
  - Typically only range, half-range (*a*) , minimum or maximum permissible values provided with no additional information
  - Rectangular distribution
  - Examples:
    - Manufacturer specifications e.g. balance



$$u(x) = \frac{a}{\sqrt{3}}$$

Type B: Rectangular distribution

- **Example:**
- Standard uncertainty associated with the linearity of a mass measurement of 5.0 g:

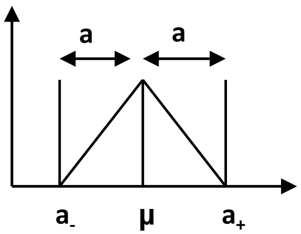
- Value:
  - m = 5.0 g
- Standard Uncertainty:

$$u(x) = \frac{a}{\sqrt{3}} = \frac{0.0002}{\sqrt{3}} = 0.0012g$$

Model		224	124	613	513	313	213	6102	5102	3102	2102	1102	612
Weighing capacity	g	220	120	610	510	310	210	6,100	5,100	3,100	2,100	1,100	610
Readability	mg	0.1	0.1	1	1	1	1	10	10	10	10	10	10
Repeatability	mg	0.1	0.1	1	1	1	1	10	10	10	10	10	10
Linearity	mg	0.2	0.2	2	2	2	2	20	20	20	20	20	20
Min. sample weight according to USP, typical	g	0.12	0.12	1.5	1.5	1.5	1.5	12	12	12	12	12	12

Quantify individual uncertainties

- Type B:
  - Typically only range, half-range (*a*) , minimum or maximum permissible values provided with indication that central value is more likely
  - Triangular distribution
  - Examples:
    - Calibration of glassware, e.g. volumetric flasks, pipettes, burettes



$$u(x) = \frac{a}{\sqrt{6}}$$

Combining uncertainties

- Uncertainties must be in same unit, or converted to relative units
- Individual uncertainties (EURACHEM):
  - Rule 1:
    - Individual uncertainties are in the same unit
  - Rule 2
    - For models involving only a product or quotient
- Combine:
  - Continue combining employing rule 1 and 2 until you obtain the combined uncertainty of the result

Combining uncertainties

- Rule 1:
  - For models involving only a sum or difference of quantities, *e.g.*

$$y = (p + q - r..)$$

the combined standard uncertainty *uc*(*y*) is given by:

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

Example: Rule 1

- Mass by Difference:

$$m_{(sample)} = m_{(boat\ +\ sample)} - m_{(boat)}$$

$$u_c\left(m_{(sample)}\right) = \sqrt{u\left(m_{(boat\ +\ sample)}\right)^2 + u\left(m_{(boat)}\right)^2}$$

- Combining individual mass contributions:

$$\boldsymbol{u_c(m) = \sqrt{u\left(m_{(readability)}\right)^2 + u\left(m_{(repeatability)}\right)^2 + u\left(m_{(linearity)}\right)^2}}$$

Model		224	124	613	513	313	213	6102	5102	3102	2102	1102	612
Weighing capacity	g	220	120	610	510	310	210	6,100	5,100	3,100	2,100	1,100	610
Readability	mg	0.1	0.1	1	1	1	1	10	10	10	10	10	10
Repeatability	mg	0.1	0.1	1	1	1	1	10	10	10	10	10	10
Linearity	mg	0.2	0.2	2	2	2	2	20	20	20	20	20	20
Min. sample weight according to USP, typical	g	0.12	0.12	1.5	1.5	1.5	1.5	12	12	12	12	12	12
Temperature limits for verified							+10°C | +30°C						

Combining uncertainties

- Rule 2
  - For models involving only a product or quotient, *e.g.*

$$y = (p \times q \times ..) \qquad \text{or} \qquad y = \frac{p}{(q \times r \times ..)}$$

the combined standard uncertainty $u_c(y)$ is given by:

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

This formula was incorrect in the original presentation

Combining uncertainties

- Rule 2
  - For models involving only a product or quotient, *e.g.*

$$y = (p \times q \times ..) \qquad \text{or} \qquad y = \frac{p}{(q \times r \times ..)}$$

the combined standard uncertainty $u_c(y)$ is given by:

$$u_c(y) = y \times \sqrt{\left(\frac{u(p)}{p}\right)^2 + \left(\frac{u(q)}{q}\right)^2 + \cdots}$$

This is the correct version of the formula.

Example: EURACHEM Rule 2

- Concentration of Mg-calibration standard:

$$Conc(Mg) = \frac{Conc(Stock) \times V(aliq)}{V(Fin\,Vol)}$$

$$u_c\big(Conc(Mg)\big) = \sqrt{\left(\frac{u(Conc_{(Stock)})}{Conc_{(Stock)}}\right)^2 + \left(\frac{u\big(V_{(aliq)}\big)}{V_{(aliq)}}\right)^2 + \left(\frac{u(V_{(Fin\,Vol)})}{V(_{Fin}\,Vol)}\right)^2}$$

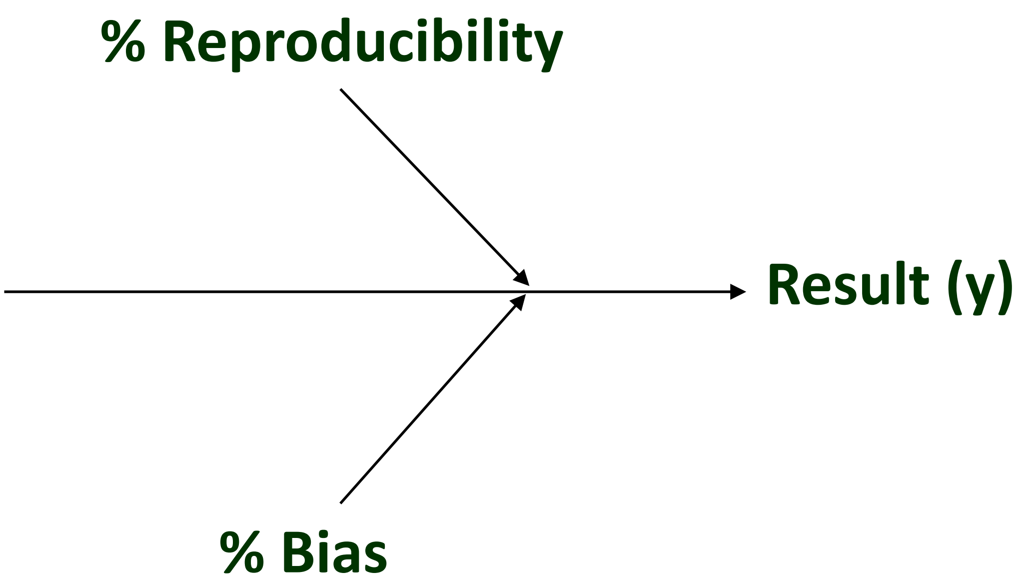
Combining uncertainties

- Uncertainties must be in same unit, or converted to relative units
- Individual uncertainties (EURACHEM):
  - Rule 1:
    - Individual uncertainties are in the same unit
  - Rule 2
    - For models involving only a product or quotient
- Combine:
  - Continue combining employing rule 1 and 2 until you obtain the combined uncertainty of the result

- Top Down Approach

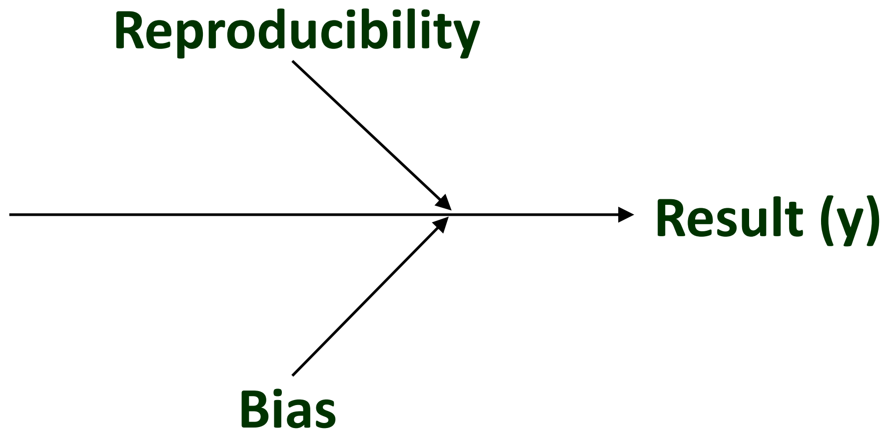
Top Down: Evaluation of UoM

- Top Down:
  - % Reproducibility
  - % Method and Laboratory Bias



$$\%u = \frac{u(x_i)}{x_i} \times 100$$

Top Down: Evaluation of UoM



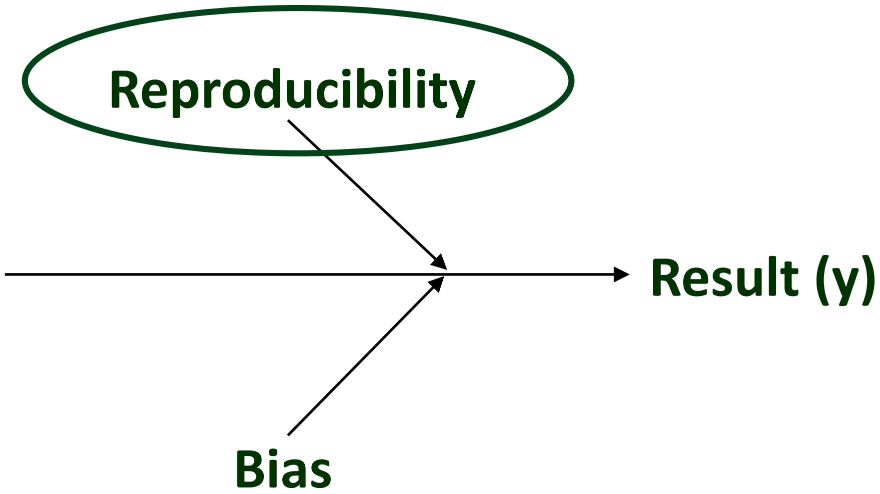
- Provided that reproducibility and bias data is representative:
  - Different stock standard solutions
  - Different batches of reagents
  - Re-calibration of instruments
  - Representative period of time – ideally 1 year
  - Minimum number of results: 50

Top Down: Evaluation of UoM

- Within laboratory reproducibility, R_w
  - Control sample covering the whole analytical process
  - Control sample not covering the whole process, matrix different

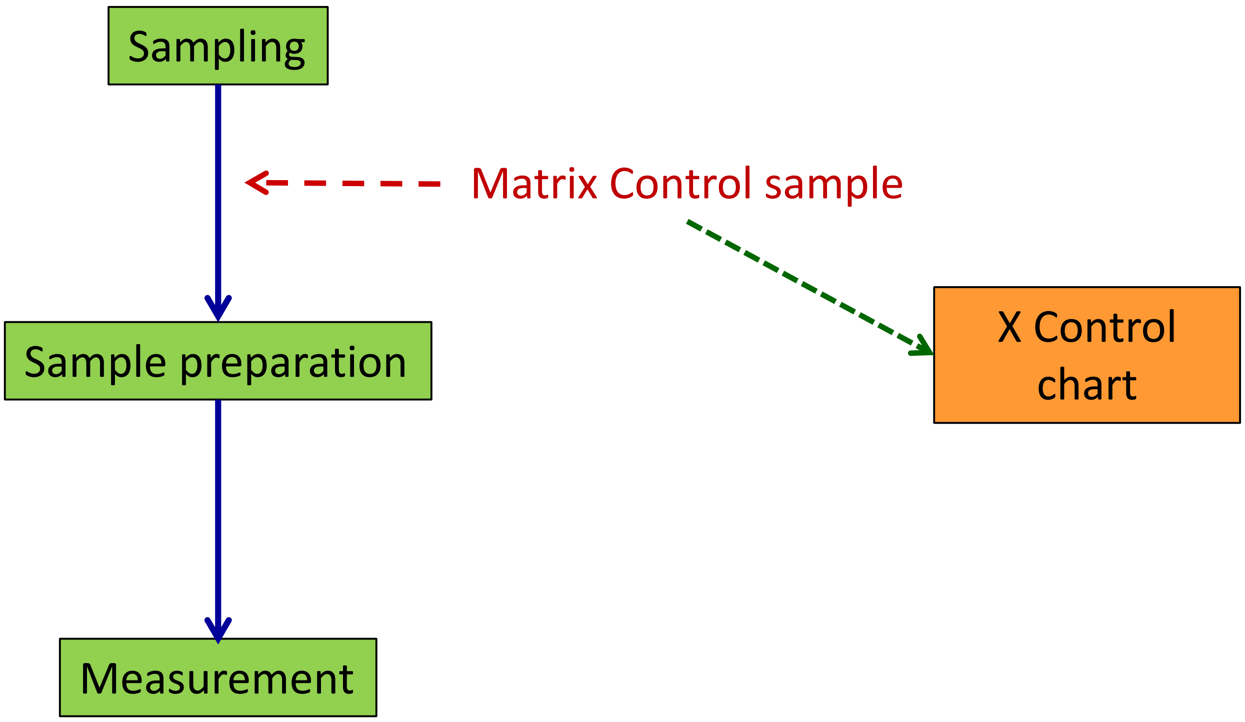
- Method Validation: Method and Laboratory Bias
  - Certified Reference Materials
  - Interlaboratory comparisons / Proficiency testing
  - Recovery experiments

Top Down: Evaluation of UoM



Within laboratory reproducibility, R_w

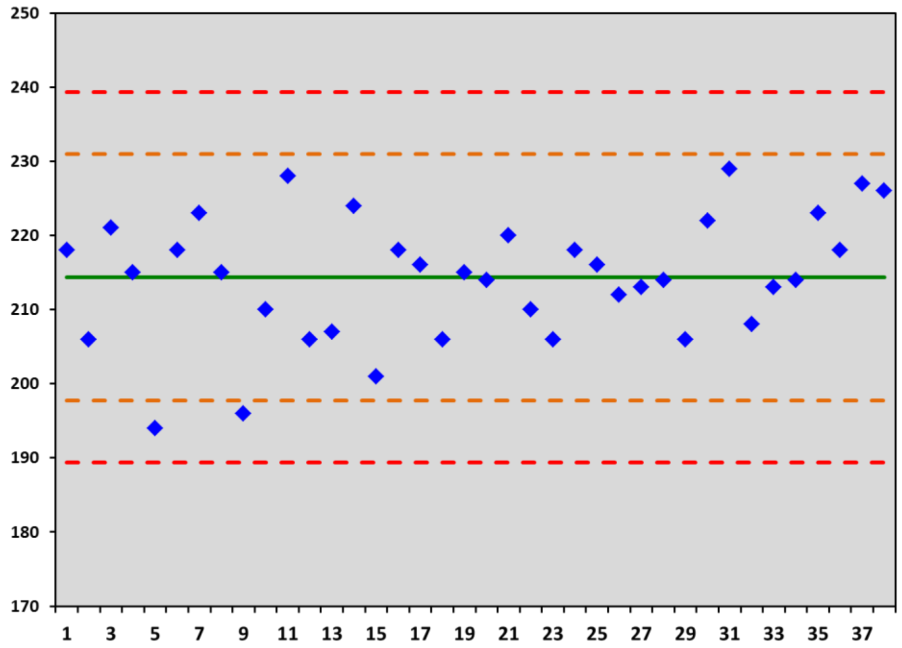
- I: Control sample covering the whole analytical process



Within laboratory reproducibility, R_w

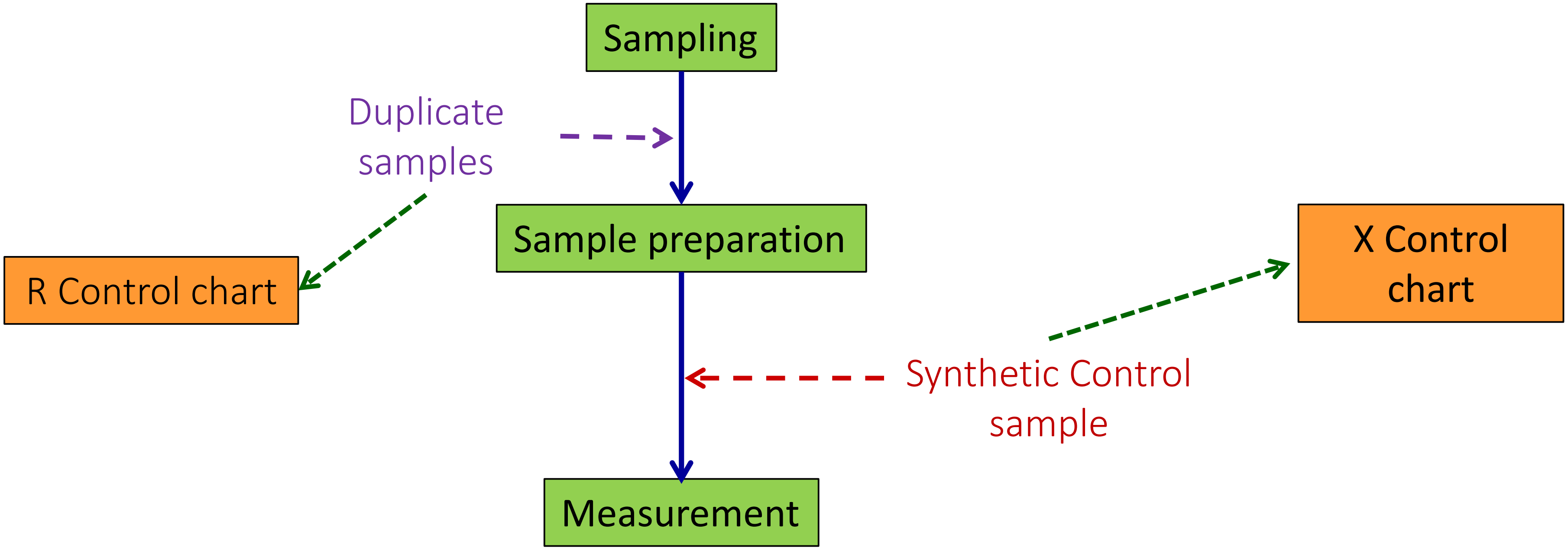
- I: Control sample covering the whole analytical process
  - Mean control chart

$$\%R_w = \frac{s_x}{\bar{x}} \times 100$$



Within laboratory reproducibility, R_w

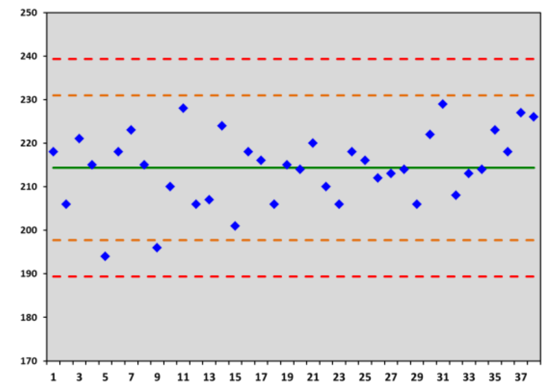
- II: Control sample not covering the whole process, matrix different



Within laboratory reproducibility, R_w

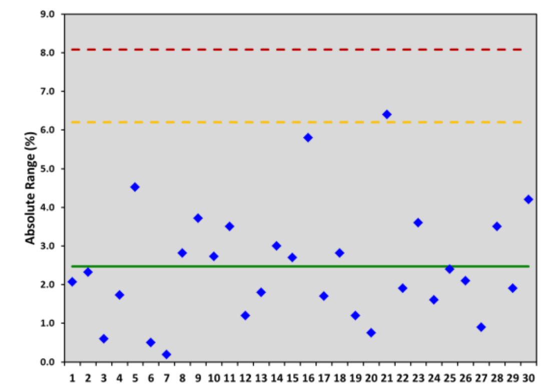
- II: Control sample not covering the whole process, matrix different
  - Mean control chart:

$$\%S_R = \frac{s_x}{\bar{x}} \times 100$$



  - Range control chart:

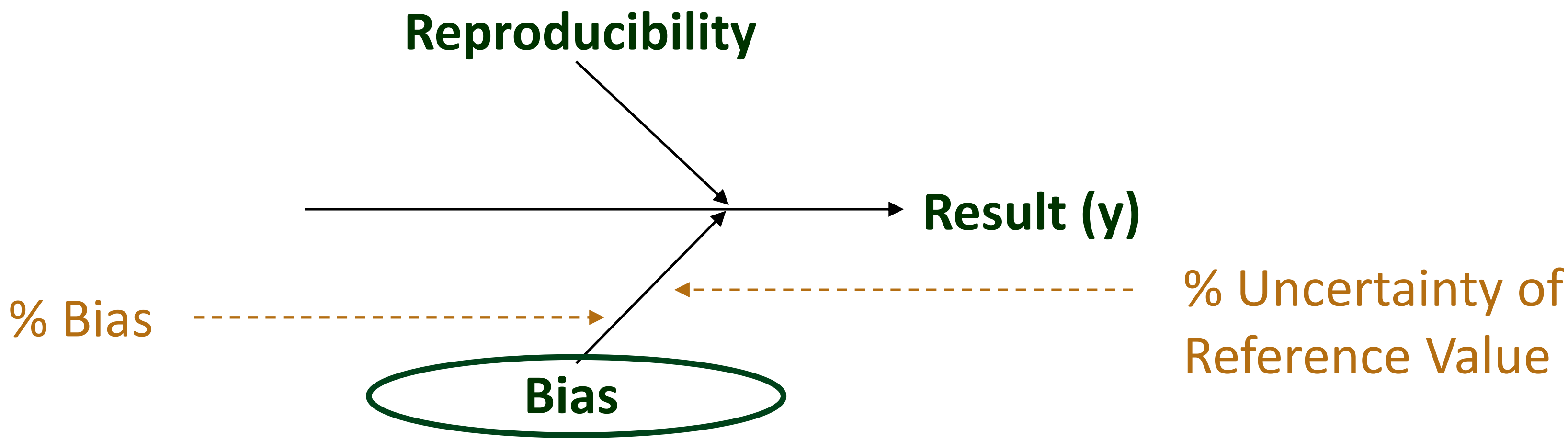
$$\%s_r = \frac{MeanRange}{d_2} \times 100$$



  - **Within laboratory reproducibility, R_w**

$$\%\boldsymbol{R_w} = \sqrt{(\%\boldsymbol{S_R})^2 + (\%\boldsymbol{S_r})^2}$$

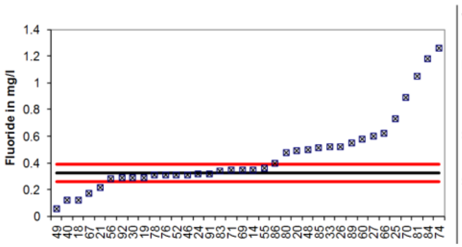
Top Down: Evaluation of UoM



Method and Laboratory Bias (u_{Bias})

- Combination of:
  - Bias, *% Bias*
  - Uncertainty associated with the reference value, *% u_c(Ref)*

- Experimentally:
  - Certified Reference Materials
  - Interlaboratory comparisons / Proficiency testing
  - Recovery experiments



Method and Laboratory Bias (u_{Bias})

- Bias, % Bias

$$\% Bias = \frac{C_{meas} - C_{Ref}}{C_{Ref}} \times 100$$

- Combined Bias (several CRMs/PTs/Recovery experiments)

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}}$$

Method and Laboratory Bias (u_{Bias})

- Uncertainty associated with the reference value, $\%u_c(Ref)$
  - CRM:
    - Expanded uncertainty, U(x): $$u(x) = \frac{U(x)}{k}$$
    - %Standard uncertainty, %u(x): $$\%u(x) = \frac{u(x)}{x} \times 100$$
  - Proficiency test:
    - Reproducibility – all participants: $\%S_R$
    - Number of participants: n
  - Recovery (EURACHEM):
    - $\%u_c(spike)$

Method and Laboratory Bias (u_{Bias})

- Combination of:
  - Bias, *% Bias*
  - Uncertainty associated with the reference value, *% u_c(Ref)*

$$u_{bias} = \sqrt{RMS_{bias}^2 + u(C_{ref})^2}$$

Top Down: Combining Uncertainty

- Calculate Combined Standard Uncertainty (u_c):
  - Combine Reproducibility and Bias components
    - Reproducibility (R_w): From control samples and other estimations
    - Bias (u_{bias}): From CRM, PT or recovery tests

$$\%u_c = \sqrt{\%u(R_w)^2 + (\%u_{bias})^2}$$

Evaluation: UoM

- Top Down:
  - Determine the expanded uncertainty (U):

$$U = k \times u_c(y)$$

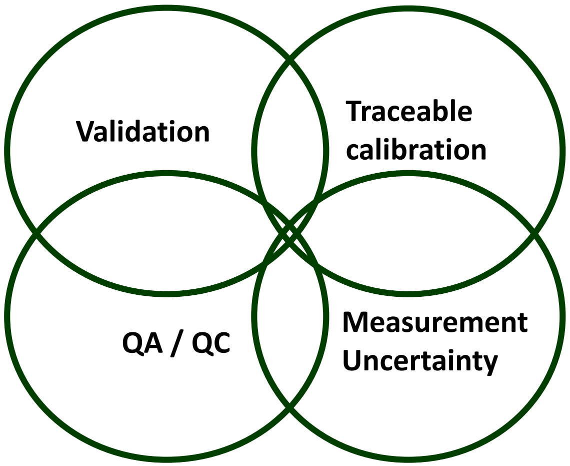
- Assume k=2 for an approximate level of confidence of 95% with assumed effective degrees of freedom > 30

Which approach to use?

- Bottom Up (GUM, EURACHEM/CITAC)
  - Mathematical model needed
  - Complex calculations
  - Smaller uncertainties
- Top Down (NORDTEST, EURACHEM/CITAC)
  - No model needed
  - Simpler combination of data already available in accredited laboratory
  - Uncertainties are larger, but perhaps more realistic?
- Fit for purpose?

Conclusions

- Method Validation
  - One part of ensuring valid, traceable results
  - Fit-for-purpose
    - Performance characteristics
    - Acceptance criteria
- Quality control
  - Internal processes
  - External processes
- Uncertainty of Measurement
  - Bottom Up
  - Top Down



Trueness: Outlier testing

- View results graphically
- Grubbs' test
  - Small number of samples
  - ISO recommended

$$G = \frac{|SuspectValue - \bar{x}|}{s}$$

  - Outlier if $G_{calc} > G_{crit}$

n	Q_{crit} CL at 90%	Q_{crit} CL at 95%	Q_{crit} CL at 99%
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

- Dixon's test (Q-test)
  - Sample sizes: n = 3 – 7

$$Q = \frac{|SuspectValue - NearestValue|}{L\,arg\,estValue - SmallestValue}$$

  - Outlier if $Q_{calc} > Q_{crit}$

Dixon Test

The Dixon test can be used to test for a single outlier in a univariate data set. This test is primarily used for small data sets (Dataplot limits the sample to be between 3 and 30).

Specifically, given a set of ordered observations Y1, Y2, ..., YN, the Dixon test is computed as follows:

Sample Size	Test for Minimum	Test for Maximum
$3 \leq N \leq 7$	$(Y_2 - Y_1)/(Y_N - Y_1)$	$(Y_N - Y_{N-1})/(Y_N - Y_1)$
$8 \leq N \leq 10$	$(Y_2 - Y_1)/(Y_{N-1} - Y_1)$	$(Y_N - Y_{N-1})/(Y_N - Y_2)$
$11 \leq N \leq 13$	$(Y_3 - Y_1)/(Y_{N-1} - Y_1)$	$(Y_N - Y_{N-2})/(Y_N - Y_2)$
$14 \leq N \leq 30$	$(Y_3 - Y_1)/(Y_{N-2} - Y_1)$	$(Y_3 - Y_1)/(Y_{N-2} - Y_1)$

https://www.itl.nist.gov/div898/software/dataplot/refman1/auxillar/dixon.htm

Dixon Test

Dixon's Q test, or just the "Q Test" is a way to find outliers in very small, normally distributed, data sets. Small data sets are usually defined as somewhere between 3 and 7 items

$$Q_{exp} = \frac{x_2 - x_1}{x_n - x_1}$$

Finding the Q statistic for different sample sizes (n) of between 8 and 30 (in Step 2 above):

8< n >10: use R_{11}:

$$r_{11} = \frac{x_2 - x_1}{x_{n-1} - x_1}$$

11< n >13: use R_{21}.

$$r_{21} = \frac{x_3 - x_1}{x_{n-1} - x_1}$$

14< n >30: use R_{22}.

$$r_{22} = \frac{x_3 - x_1}{x_{n-2} - x_1}$$

https://www.statisticshowto.datasciencecentral.com/dixons-q-test/

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- Statistics and Chemometrics for Analytical Chemistry, JN Miller, JC Miller
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- Internal Quality control, NORDTEST Report TR 569
- Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories, NORDTEST Report TR 537
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Sodium in Food: Proficiency testing scheme

Round 1	Savoury Stock Powder (Oct 2018)
Round 2	Bread (Dec 2018)
Round 3	Instant Noodles (Feb 2019)
Round 4	Fat Spread (Apr 2019)
Round 5	Flavoured Snack/Crisp (May 2019)
Round 6	Cured processed meat product (Jul 2019)

Sodium in Food: Proficiency testing scheme

Main parameter: Salt (sodium) content	
Optional Parameters:	
Iron and Zinc	Moisture
Protein	Fat
Total sugar	Cholesterol
Dietary fibre	Vitamins A, B1, B2, B3, B6, folic acid
Energy	

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We measure what matters

Thank you

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