

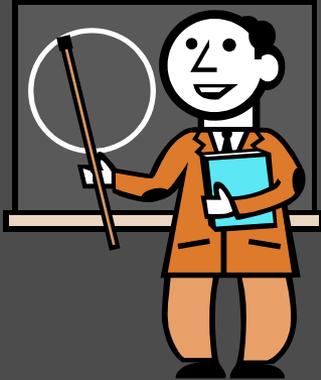


Introduction of the measurement uncertainty concept in Nestlé laboratories

Lionel Spack



Agenda



- Overview of the Nestlé Research Center
- Introduction to measurement uncertainty
- Guidelines and procedures available
- Procedure of the Nestlé Research Center
- Conclusion



Nestlé Research Center in Lausanne



- Inaugurated in 1987
- Basic knowledge in the food and life sciences
- Application in the whole Nestlé Group
- Part of the international scientific community



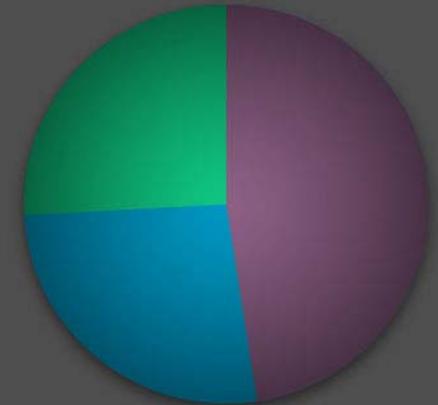
Nestlé Research Center

The Competence Base rests on three Pillars

- **Food Science and Process Research**
(Physical / Chemical Systems)

- **Bioscience**
- **Nutrition**
- **Plant Science**
(Life Sciences / Biological Systems)

- **Food Safety & Quality Assurance**
(Risk Evaluation / Analytical Methods)



Results

Science base for all product areas
Health and quality claims
Safe products



Food Quality and Safety at NRC

Implementing Quality and Safety along the Food Chain



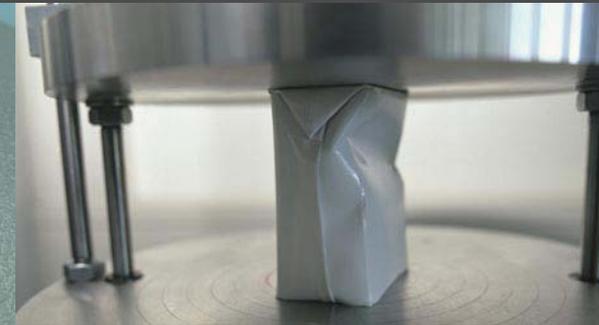
Food Safety

- Risk evaluation
- Industrial hygiene
- Contaminants
- Biomarkers



Food Analysis

- Laboratory instructions
- Authenticity
- GLP implementation
- On-line methods



Packaging

- Migration assessment
- Off-flavours
- Novel materials
- Active packaging



Development of analytical methods



- Quality and safety control
- Verification of specifications
- Control of raw material
- Fit for purpose and validated
- Fulfill the ISO 17025 requirements



**Measurement
Uncertainty
Estimation**



What are we talking about?

- Measurement uncertainty expresses the degree of doubt associated with a measurement
- There is always experimental variations when we make a measurement
- A result should be reported with a tolerance interval

Result: $28 \pm 4\text{mg}$



Requirement for accredited laboratories

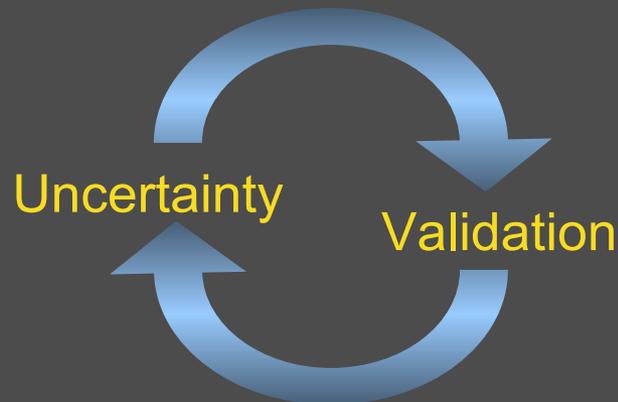
- ISO/IEC 17025: 5.4.6 Estimation of uncertainty of measurement

5.4.6.2 Test laboratories should have and should apply procedures for estimating uncertainty of measurement. In certain cases, the nature of the test method may preclude rigorous, metrologically and statistically valid calculation of uncertainty of measurement. In these cases the laboratory should at least attempt to identify all the components of uncertainty and make a reasonable estimation, and should ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation should be based on knowledge of the performance of the method and on the measurement scope and should make use of, for example, previous experience and validation data.



Use of measurement uncertainty (MU)

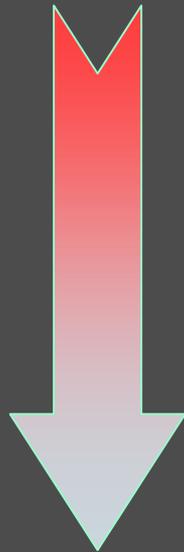
- To enable a result to be interpreted with respect to a limit
- To compare results obtained on the same material from different laboratories
- To reveal those part of a method having the greatest variability during method validation





Guidelines Available

General

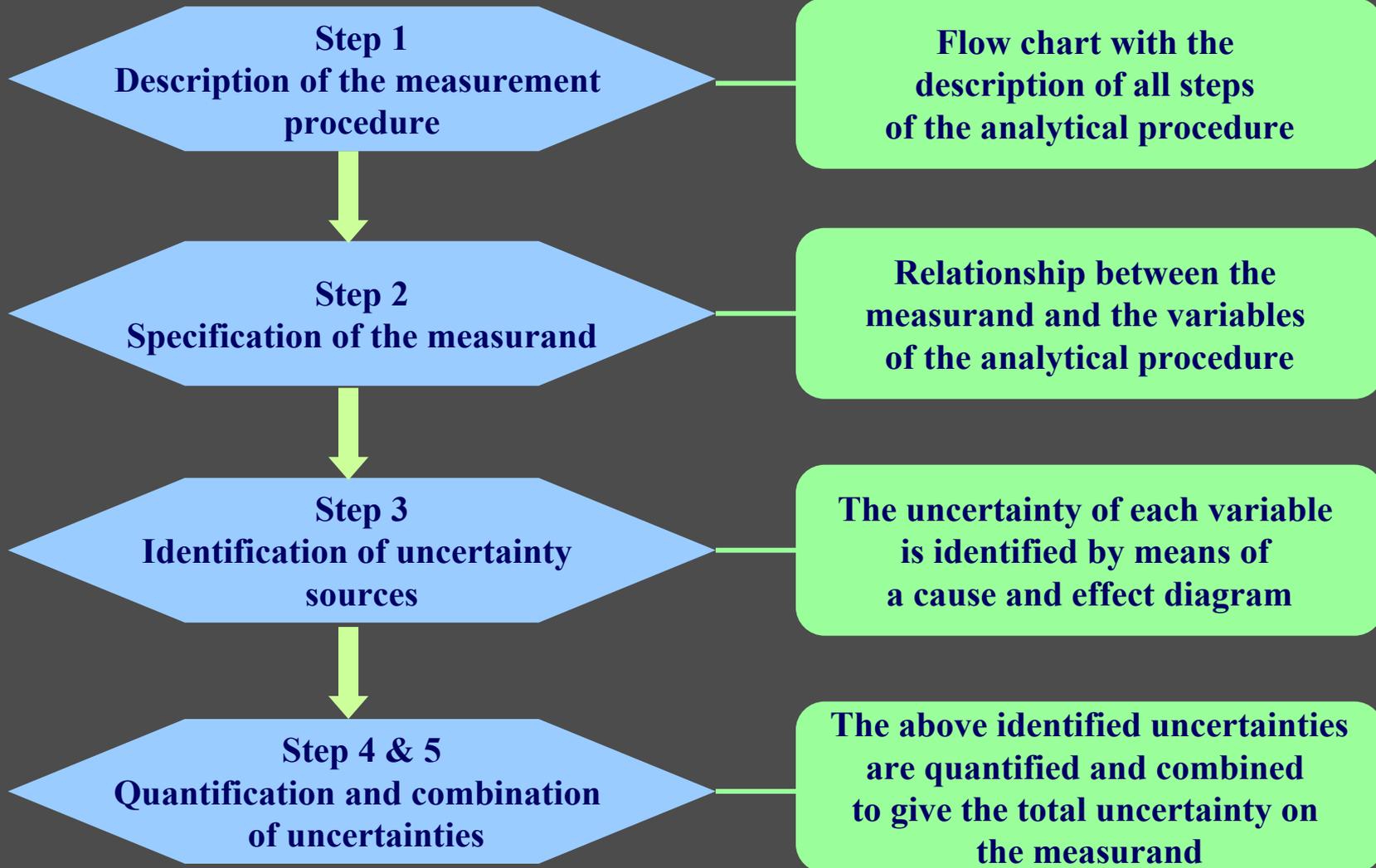


Specific

- ISO Guide for the expression of uncertainty
- Eurachem Guide for quantifying uncertainty in analytical chemistry
- VAM Protocol for uncertainty evaluation from validation data

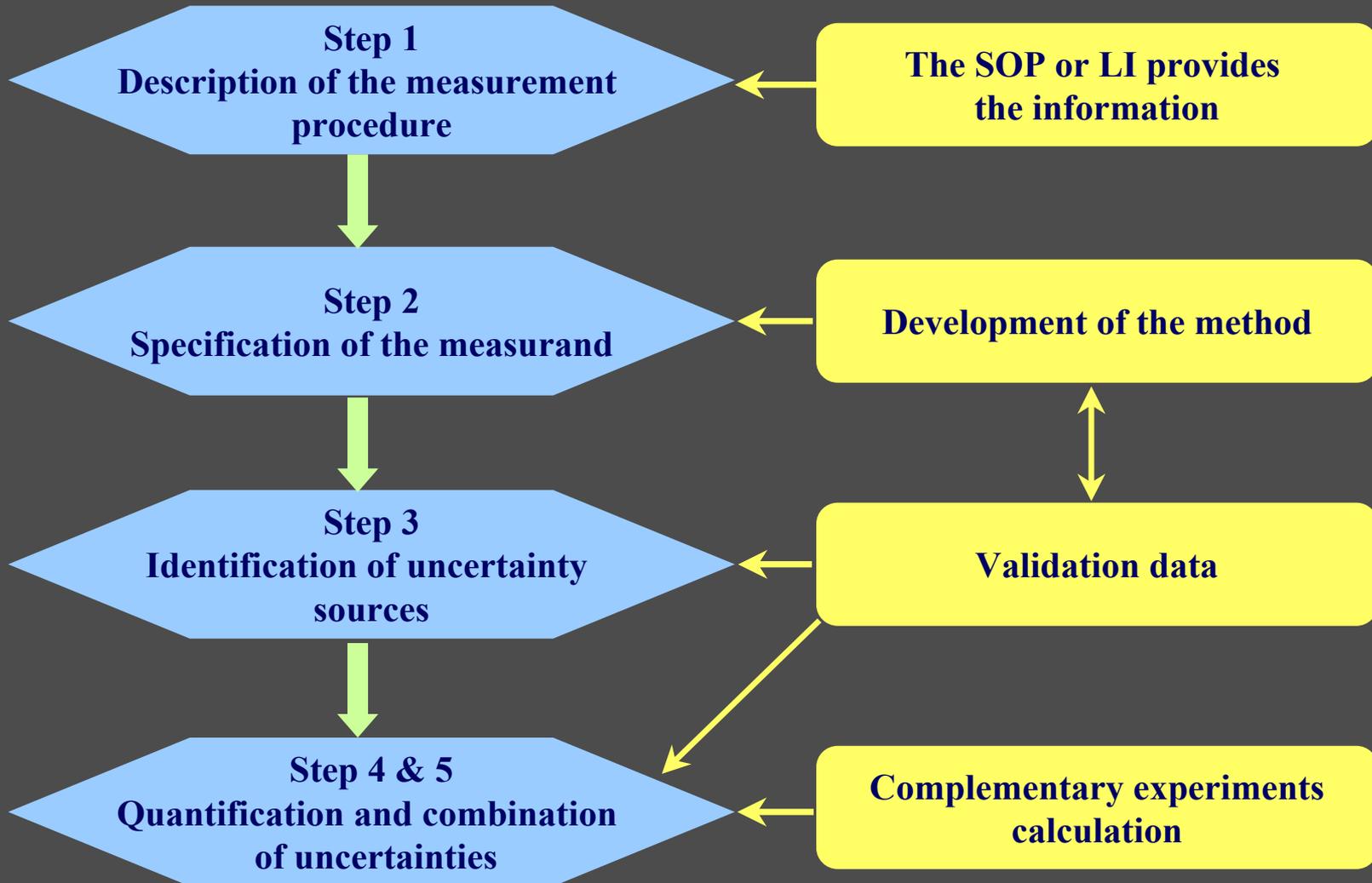


General Approach of MU



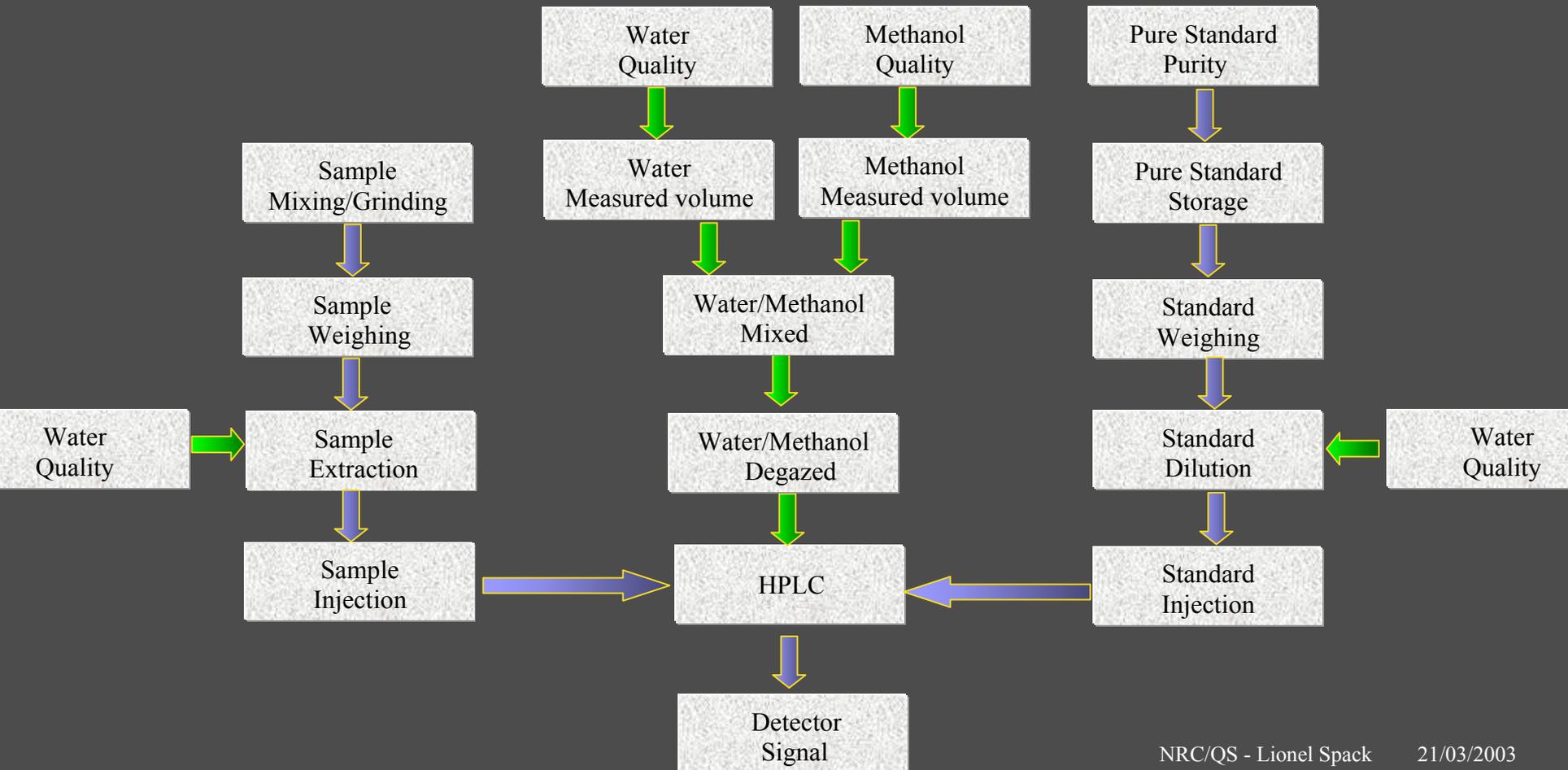


Nestlé Approach





Example: determination of Caffeine by HPLC





Equation for Caffeine by HPLC

$$\frac{A_s}{A_0} \cdot \frac{C_0}{m_s \cdot 10^6} \cdot V_s \cdot \frac{1}{\text{Rec}} = \text{Caffeine content (g/100g)}$$

A_s

Peak Area of Sample

C_0

Concentration of the standard [mg/l]

V_s

Volume of Sample Solution [ml]

A_0

Peak Area of Standard

m_s

Mass of the sample [g]

10^6

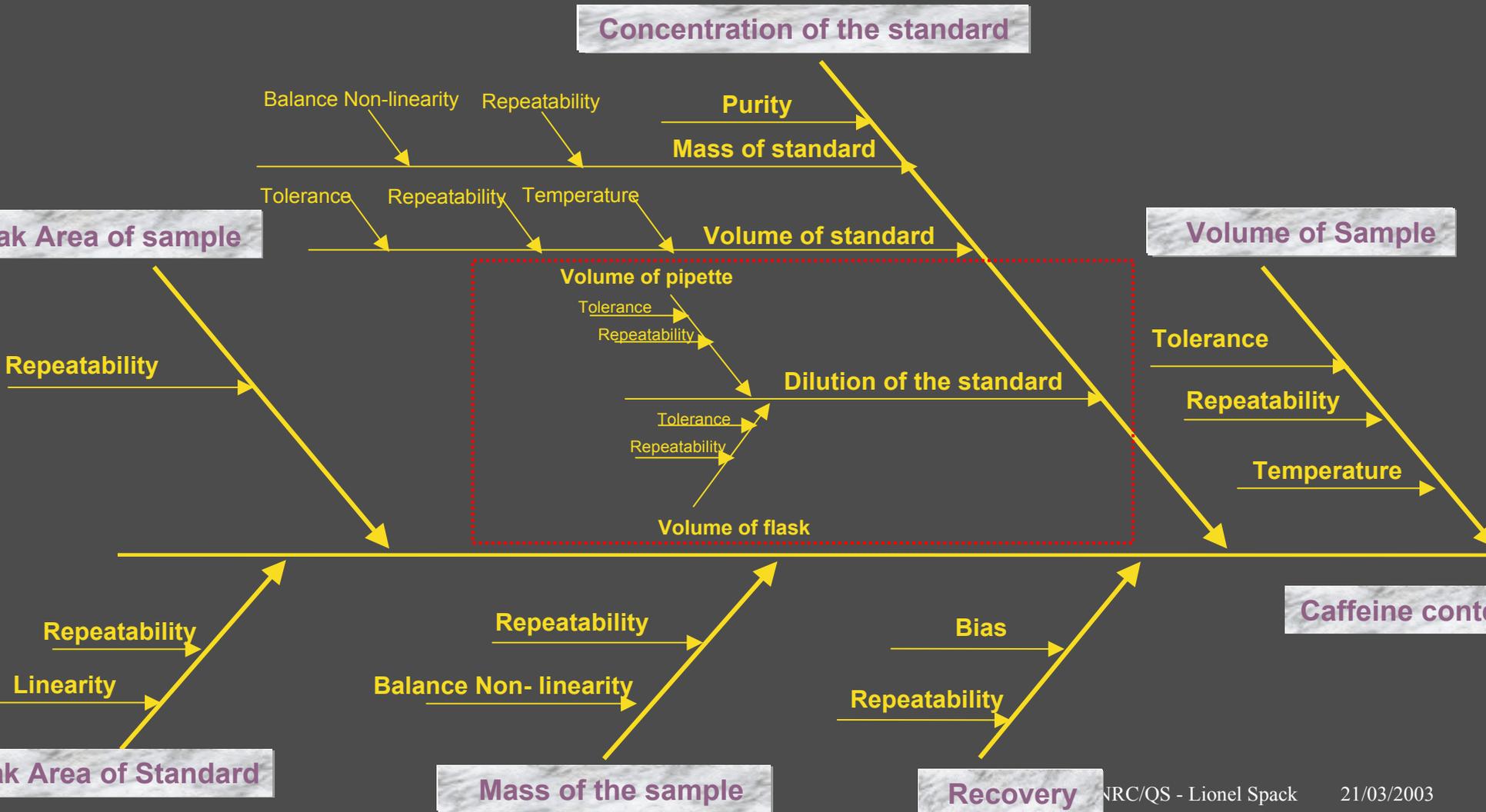
Conversion factor

Rec

Recovery



Cause and effect diagram for Caffeine by HPLC





Validation data available for Caffeine by HPLC

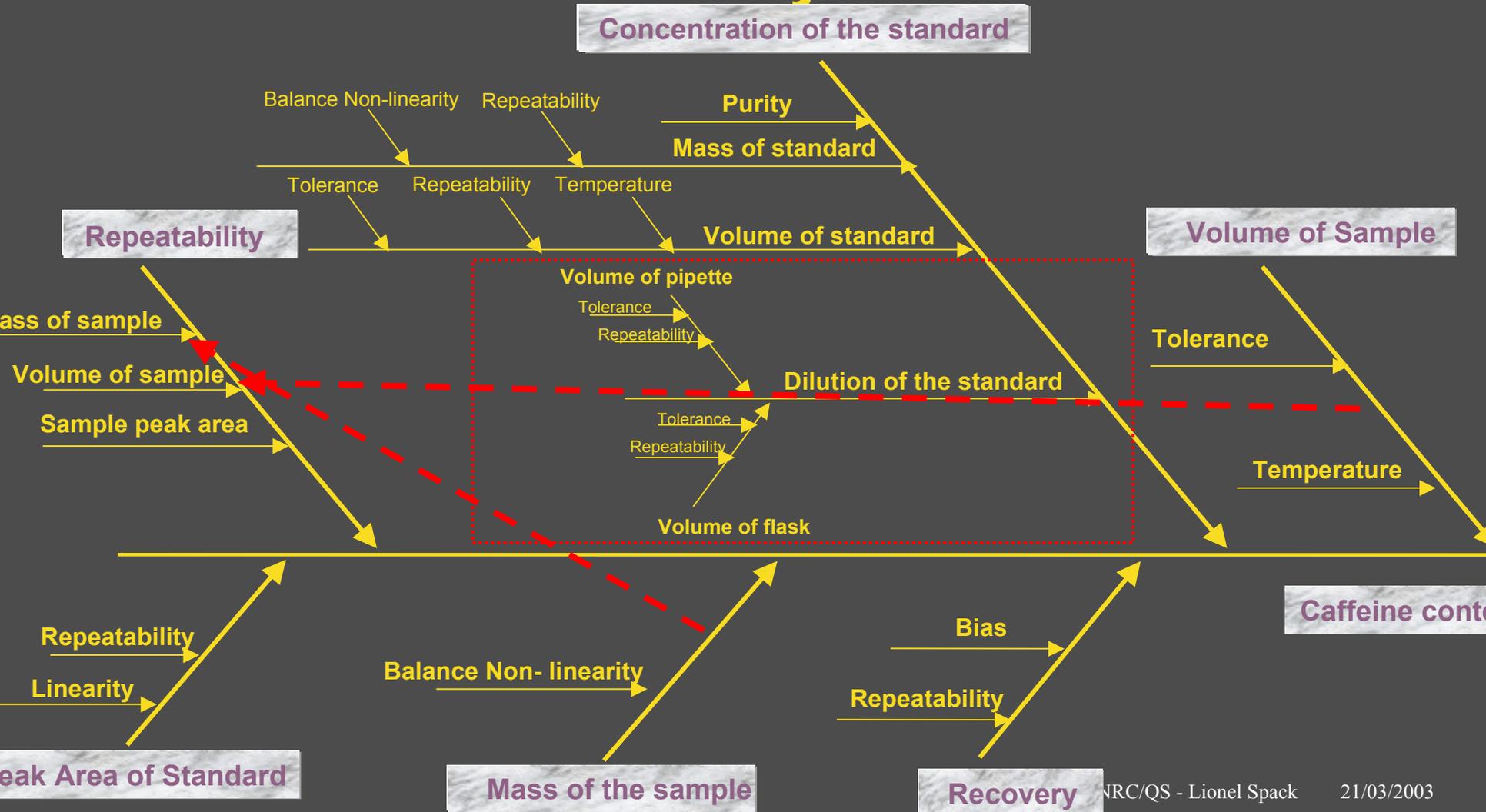
- Precision study: repeatability conditions
- Trueness study: experiments with references
- Analysis in duplicates



Refining the cause and effect diagram
• Repeatability branch



Refined cause and effect diagram for Caffeine by HPLC:



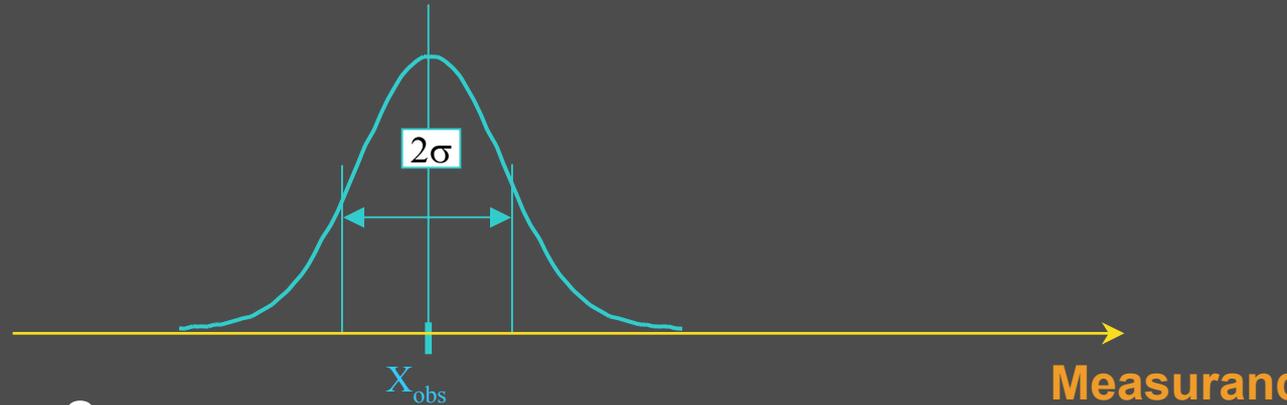


Uncertainty budget for Caffeine by HPLC

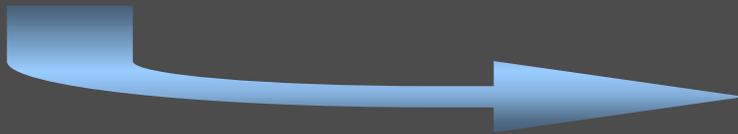
Variable Name	Variable (x) Value	Units	Standard Uncertainty [u(x)]	Relative Standard Uncertainty [u(x)/x]
Repeatability	(y) value			
Recovery				
Peak Area of Standard				
Standard concentration				
Volume of Sample				
Mass of Sample				
Combined standard uncertainty u(y)	(y) value			



Precision study



- Repeatability conditions ?
- Intermediate reproducibility conditions ?
- Reproducibility conditions ?



- ✎ From duplicates ?
- ✎ From set of data at different levels
- ✎ Concentration dependant ?
- ✎ Matrix dependant ?



Precision values for Caffeine by HPLC:

- The repeatability limits for a confidence level of 95% are:
 $r = 0.10\text{g}/100\text{g}$ for non decaffeinated products
 $r = 0.02\text{g}/100\text{g}$ for decaffeinated products
- The precision values expressed as standard deviation are:

For non decaffeinated products:

$$SD(r) = \frac{0.10}{2.77} = 0.036\text{g}/100\text{g}$$

For decaffeinated products:

$$SD(r) = \frac{0.02}{2.77} = 0.0072\text{g}/100\text{g}$$



Repeatability values in budget

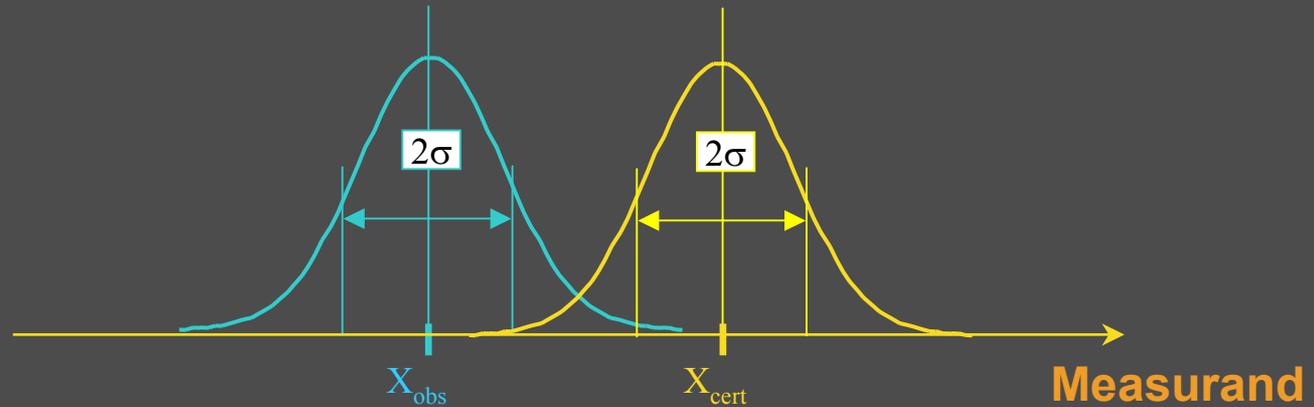
Variable Name	Variable (x) Value	Units	Standard Uncertainty [u(x)]	Relative Standard Uncertainty [u(x)/x]
Repeatability	0.04^a 2.81^b	g/100g	0.0072 0.036	0.18 0.012
Recovery				
Peak Area of Standard				
Standard concentration				
Volume of Sample				
Mass of Sample				
Combined standard uncertainty u(y)	0.04 2.81	g/100g		

a: decaffeinated sample

b: non decaffeinated sample



Trueness study



- Reference material/samples ?
- Reference method ?

- ☞ True within repeatability confidence interval ?
- ☞ Concentration dependant
- ☞ Matrix dependant ?
- ☞ Correction factor ?
- ☞ Correction in terms of MU ?



Trueness data for Caffeine by HPLC

- **Non decaffeinated samples**

The mean recovery obtained with 6 repetitions of a test sample is 1.001 with a standard deviation of 0.001. A t-test shows that the recovery is not different from 1.0. No correction.

- **Decaffeinated samples**

The mean recovery obtained with 4 repetitions of a test sample is 0.93 with a standard deviation of 0.02. A t-test shows that the recovery is different from 1.0. A correction is necessary. No correction factor is applied, therefore the standard deviation on recovery must be “extended”.



Recovery values in budget

Variable Name	Variable (x) Value	Units	Standard Uncertainty [u(x)]	Relative Standard Uncertainty [u(x)/x]
Repeatability	0.04 ^a	g/100g	0.0072	0.18
	2.81 ^b		0.036	0.012
Recovery	1		0.042	0.037
	1.001		0.013	0.008
Peak Area of Standard				
Standard concentration				
Volume of Sample				
Mass of Sample				
Combined standard uncertainty u(y)	0.04 2.81	g/100g		

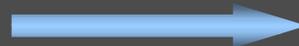
a: decaffeinated sample

b: non decaffeinated sample



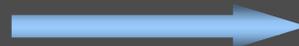
Additional uncertainty sources

• Calibration model ?



👉 Error function ?

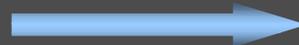
• Dilution scheme ?



👉 Calculation ?

• Biases ?

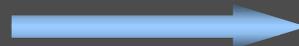
- Balance
- Glassware
- Instruments



👉 Specifications ?

• Environmental factors ?

- Temperature
- pH
- Flow rate
- ...



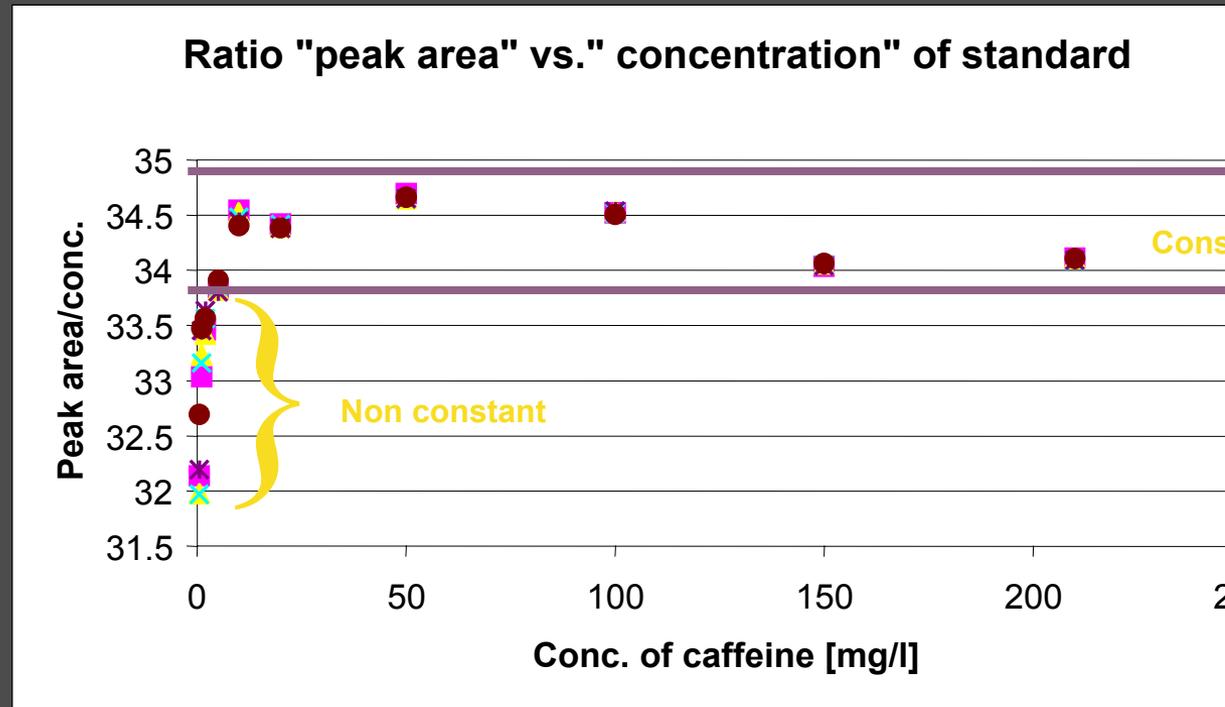
👉 Ruggedness study ?



Calibration model for Caffeine by HPLC

Simple relationship

- Plot the instrumental signal/mass injected against the mass injected
- Define the linear domain
- For this domain calculate the standard deviation of the signal.
- Use this standard deviation for the uncertainty linked to the standard.





Calibration value in budget

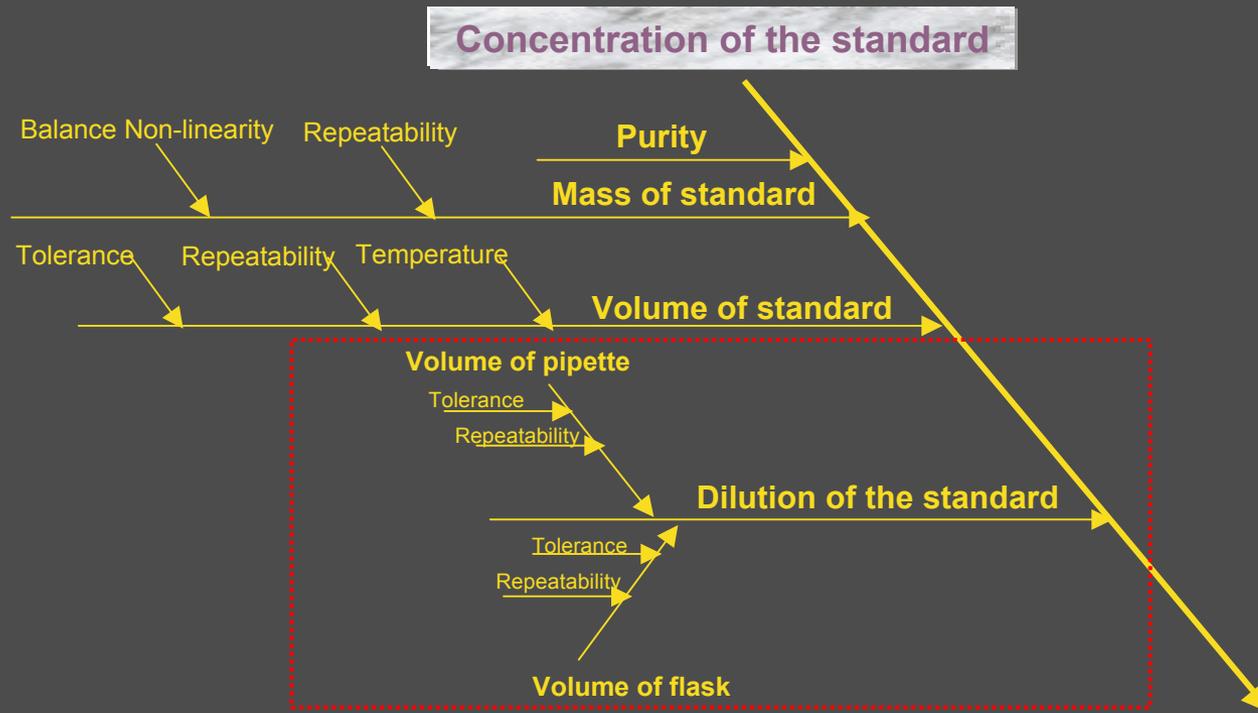
Variable Name	Variable (x) Value	Units	Standard Uncertainty [u(x)]	Relative Standard Uncertainty [u(x)/x]
Repeatability	0.04 ^a	g/100g	0.0072	0.18
	2.81 ^b		0.036	0.012
Recovery	1		0.037	0.037
	1		0.008	0.008
Peak Area of Standard	344		4.12	0.012
	3450		41.4	0.012
Standard concentration				
Volume of Sample				
Mass of Sample				
Combined standard uncertainty u(y)	0.04 2.81	g/100g		

a: decaffeinated sample

b: non decaffeinated sample



Preparation of standard solutions





Total uncertainty in budget

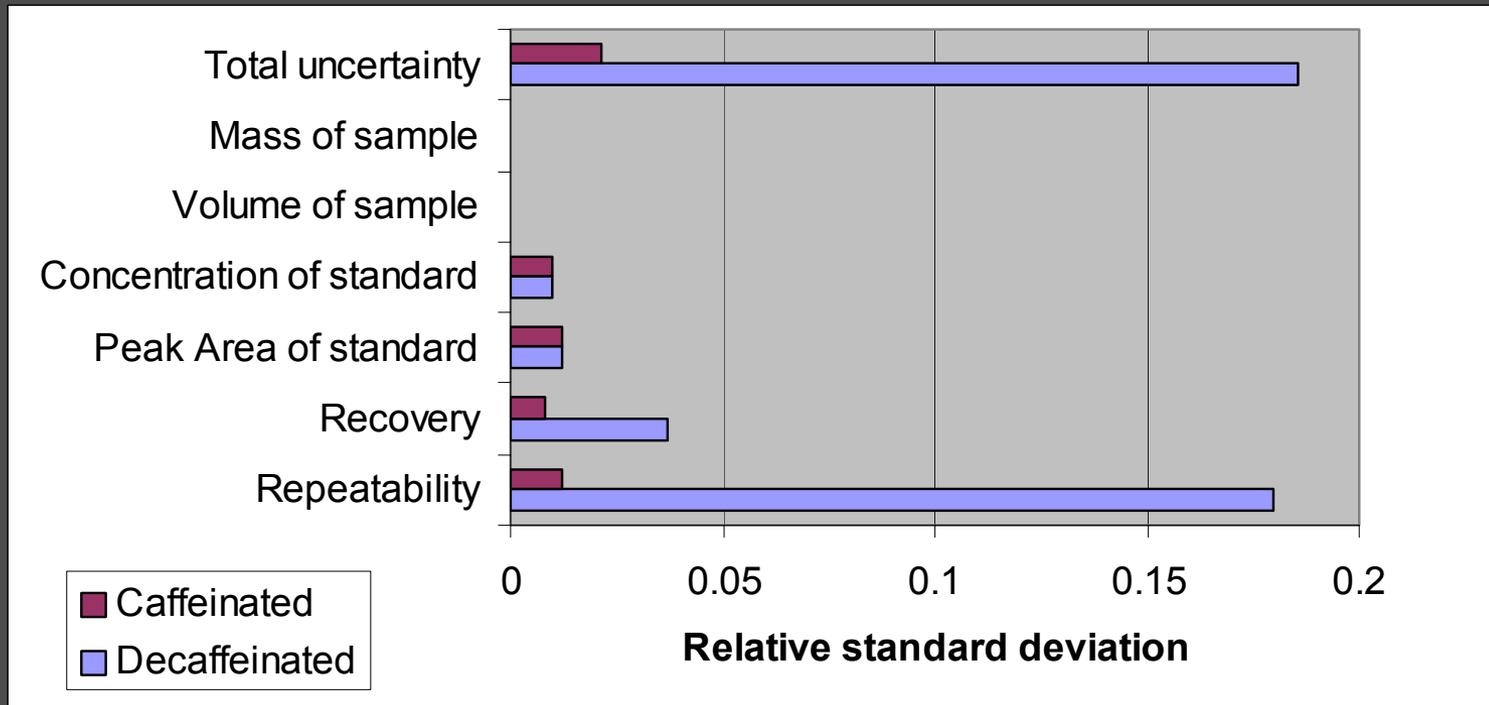
Variable Name	Variable (x) Value	Units	Standard Uncertainty [u(x)]	Relative Standard Uncertainty [u(x)/x]
Repeatability	0.04 ^a	g/100g	0.0072	0.18
	2.81 ^b		0.036	0.012
Recovery	1		0.037	0.037
	1		0.008	0.008
Peak Area of Standard	344		4.12	0.012
	3450		41.4	0.012
Standard concentration	10	mg/L	1.12	0.011
	100		0.11	0.011
Volume of Sample	0.25	L	0.00019	0.0008
Mass of Sample	4.0	g	0.00016	0.00004
	0.5		0.00016	0.00032
Combined standard uncertainty u(y)	0.04	g/100g	0.0074	0.185
	2.81		0.059	0.021



a: decaffeinated sample
b: non decaffeinated sample



Graphical presentation of measurement uncertainty





Expression of total uncertainty as an interval

Results : Caffeine _ content $\pm U(\text{Caffeine _ content})$

Where U is called **expanded uncertainty** and corresponds to the standard uncertainty $u(\text{Caffeine_content})$ multiplied by a coverage factor $k=2$ (95% Confidence Level).

Decaffeinated sample:

Results : $0.04 \pm (2 \times 0.0074) \Rightarrow \pm 0.015 \text{ g} / 100 \text{ g}$

Non decaffeinated sample:

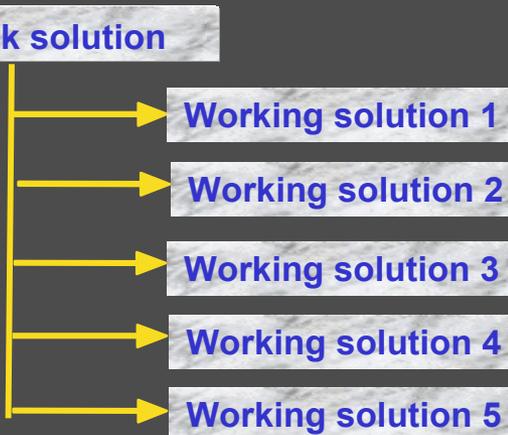
Results : $2.81 \pm (2 \times 0.059) \Rightarrow \pm 0.12 \text{ g} / 100 \text{ g}$



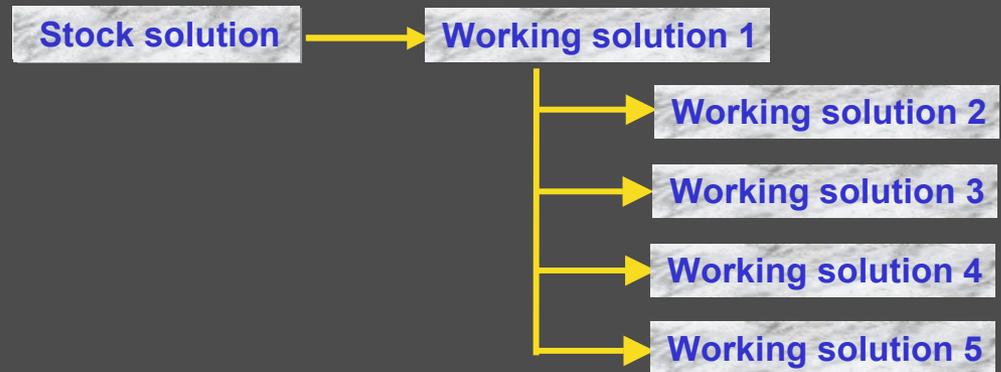
What can be simplified ?

Repeatability in %	Possible simplification
$RSDr < 1\%$	Nothing
$1\% \leq RSDr \leq 5\%$	Mass of sample Volume of sample Sample dilution
$5\% \leq RSDr \leq 10\%$	Simple standard preparation: 1 step
$10\% \leq RSDr \leq 15\%$	Dilution scheme 1
$15\% \leq RSDr \leq 20\%$	Dilution scheme 2

Scheme 1



Scheme 2

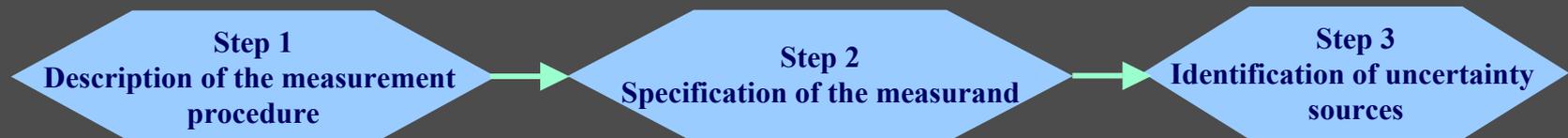




Selection of a procedure for the estimation of measurement uncertainty

- For widely used methods (**In-house or Official**) intermediate reproducibility (iR) can be used as measurement uncertainty
- For new **Official methods**, the laboratory has to check that it fulfills the precision and trueness limits defined in the method. The reproducibility (R) given by the method can be used as measurement uncertainty
- For new or less used **In-house** methods the measurement uncertainty estimation has to be completely performed

In all cases the three first steps are presented





Organisation in Network

- **Nestlé Research Center:**
 - Develops and publishes procedures
 - Calculate examples
- **The Regional Laboratories:**
 - Perform more analyzes
 - Provide data like (SD_{iR})





Conclusion

- The approach is systematic and easy to understand and to verify
- It is pragmatic and based on validation data
- It can be integrated in validation study
- It can be transferred to other laboratories



Fulfills ISO 17025



Thank you for your attention !!!

