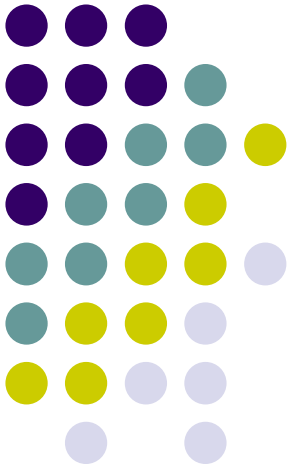
Validation of Analytical Methods

Evsevios Hadjicostas



Introduction

- The importance of method validation
- Use of the information in this chapter
- Who should be aware of this chapter

Contents

- The whats, whys, whens and hows of method validation
- The method performance parameters
- How to use validated methods

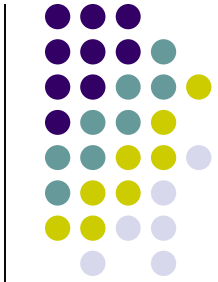
What is Method Validation?

- Method validation is the process of proving that an analytical method is acceptable for its intended purpose

What is Method Validation?

- Method performance parameters are determined using equipment that is:
  - Within specification
  - Working correctly
  - Adequately calibrated
- Competent operators
- Method validation and method development

Why is Method Validation Necessary?

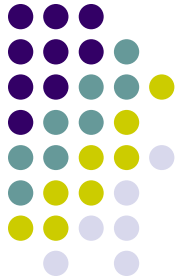


- To increase the value of test results
- To justify customer's trust
- To trace criminals
- To prove what we claim is true

Examples

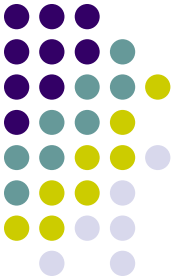
- To value goods for trade purposes
- To support health care
- To check the quality of drinking water

The Professional Duty of the Analytical Chemist



- To increase reliability of laboratory results
- To increase trust of laboratory customers
- To prove the truth

When should Methods be Validated

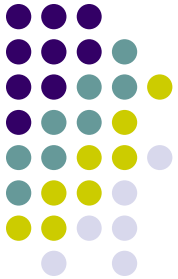


- New method development
- Revision of established methods
- When established methods are used in different laboratories/different analysts etc.
- QC indicates method changes
- Comparison of methods

How should Methods be Validated
Who Carries out Method Validation (I)

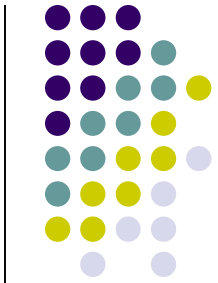
- Validation in a group of laboratories
  - Collaborative studies
  - Inter-laboratory comparisons

How should Methods be Validated
Who Carries out Method Validation (II)



- Validation in a single laboratory
  - Comparisons with CRMs
  - Comparisons with other methods that are validated

How should Methods be Validated
The Analytical Requirement (I)

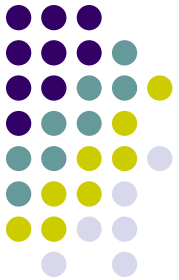


- What are the analytes of interest?
- What are the expected concentration levels?
- Are there any interferences?
- How was the sampling done?

How should Methods be Validated The Analytical Requirement (II)

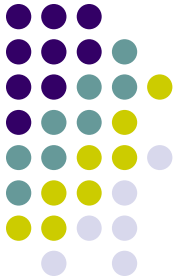
- Which method is the most suitable?
- What degree of validation is required?
- How the method will be used?

How should Methods be Validated
Method Development



- The one extreme: few sketchy ideas
- The other extreme: minor changes

Validated
To What Degree Validation is Required



- Time and cost constrains
- Customers’ requirements
- Existing experience
- Compatibility with other similar methods

How should Methods be Validated
What Degree of Validation is Required

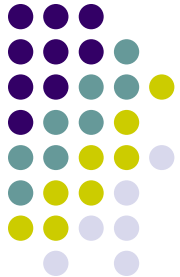
Category of the method	Action
Interlaboratory tested	Precision, trueness
Interlaboratory tested but it applies with different material, different instrument	Precision, trueness, limit of detection, selectivity
Established but not tested	Many
From bibliography, with reference to performance characteristics	Many
From bibliography, without reference to performance characteristics	Many
In-house method	Full validation

How should Methods be Validated?

What to Check (Performance Characteristics)

- Selectivity/Specificity
- Limit of Detection
- Limit of Quantitation
- Linearity
- Accuracy
- Trueness
- Sensitivity
- Ruggedness (or Robustness)
- Recovery

Identity and Selectivity/Specificity

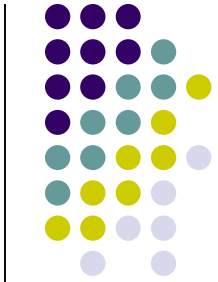


- Identity: Signal to be attributed to the analyte
  - GLC (change column/polarity), GC/MS, Infra-red
- Selectivity: The ability of the method to determine accurately the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test.
- Specificity is a state of perfect selectivity

Identity and Selectivity/Specificity

- Confirmation versus repeatability
  - Confirmation: Measure by more than one technique
  - Repeatability: Measure several times by one technique

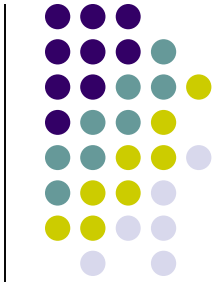
Identity and Selectivity/Specificity



- How to establish selectivity:

  Compare the response of the analyte in a test mixture with the response of a solution containing only the analyte.

Identity and Selectivity/Specificity



- The procedure to establish selectivity:
  - Analyze samples and reference materials
  - Assess the ability of the methods to confirm identity and measure the analyte
  - Choose the more appropriate method.
  - Analyze samples
  - Examine the effect of interferences

Limit of Detection (LoD)

- LoD:The lowest concentration of analyte in a sample that can be detected
  - LoD = $B+3S_0$ or $0+3S_0$
    (for fortified samples;
    typically, three times the noise level)
    - B=Blank
    - S_0=standard deviation of 10 measurements

Expression of the LoD

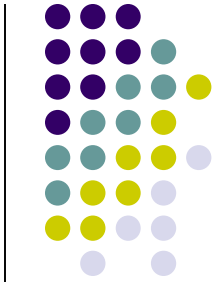
- Analyze
  - 10 independent sample blanks and get the mean sample blank value (B) **or**
  - 10 independent sample blanks fortified at lowest acceptable concentration.
- Express LoD as the analyte concentration corresponding to
  - B+3s or
  - 0+3s

  (s being the sample standard deviation).

Limit of Quantitation (LoQ)

- The Limit of Quantitation is the content which is equal or grader than the lowest concentration point on the calibration curve (i.e. what level can be measured)
  - $LoQ = B+10S_0$

Limit of Detection (LoD)
Limit of Quantitation (LoQ)



- Graphical method
  - Six measurements in 3 concentration levels
  - Std.dev. for each one level
  - S=f(C)
  - S_0=intercept
  - LoD = $3S_0$
- Signal to Noise ratio S/N=3 (for the LoD)
- Signal to Noise ratio S/N=10 (for the LoQ)

Linearity and Working Range

The ability of the method to obtain test results which are proportional to the concentration of the analyte

Linearity and Working Range

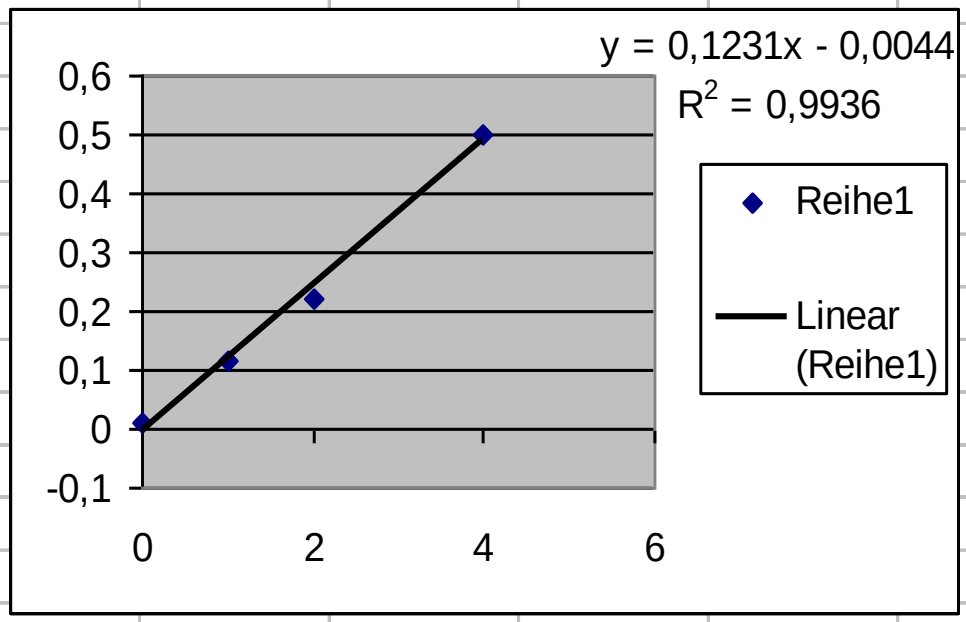
- Correlation coefficient, sufficient when
  - $r^2 > 0.99$
  - or 0.98 for very low concentrations
- Criterion No 1: $R=kC^n$
  - $\log R = n \log C + \log k$, $\text{Log}R = f(\log C)$, n=slope $1.1 \geq n \geq 0.9$
  - (R:Response of the blank, k sens.,C conc, n coeff.)
- Criterion No 2:

$$\frac{R_s - R_{bl}}{c} = f(c)$$

  - Response of the sample minus the response of the blank divided by the concentration as a function of the concentration value

Regression Line, r^2

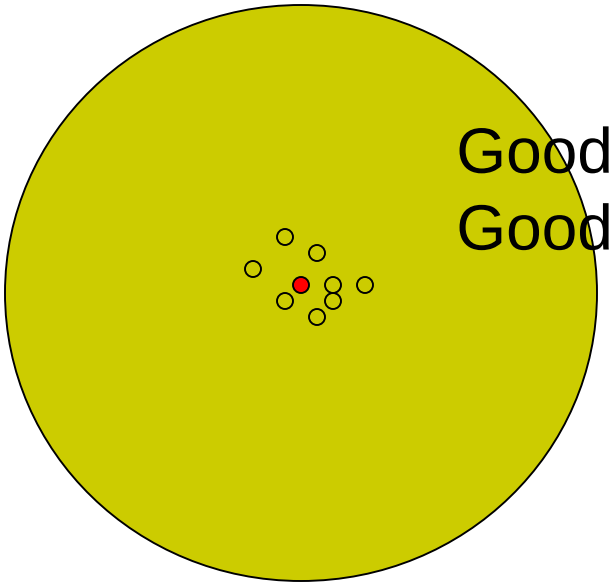
0	0,01
1	0,114
2	0,22
4	0,5



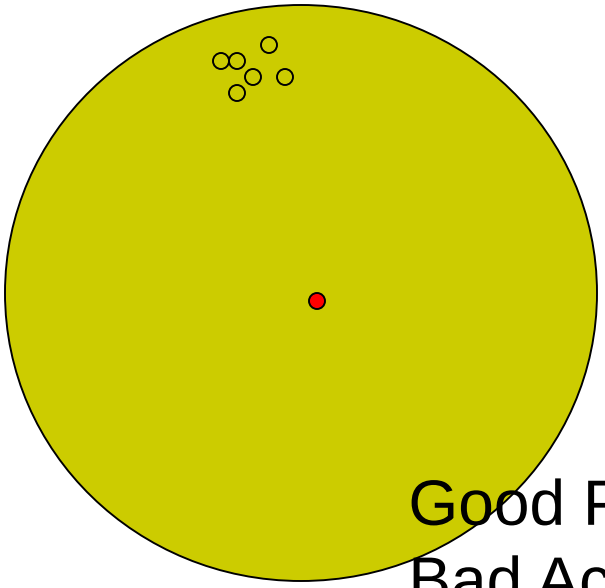
Visual inspection is required

Accuracy / Trueness

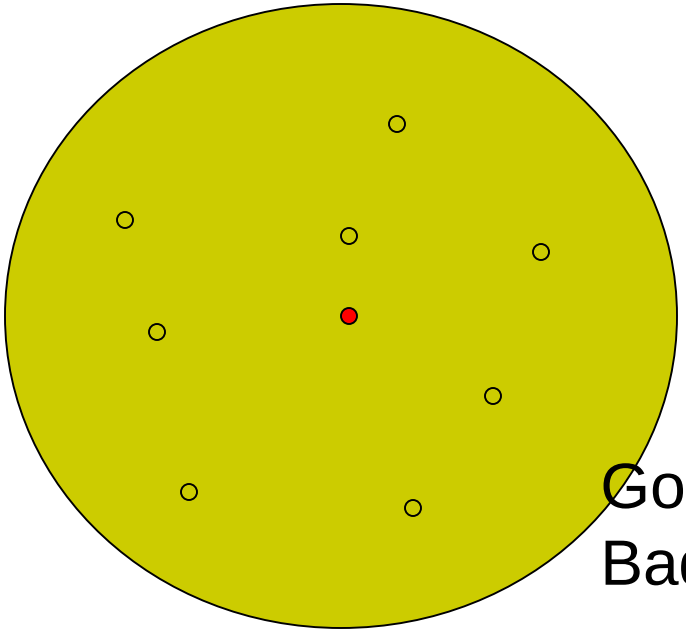
- Accuracy: the closeness of a result to a true value (=trueness+precision).
- Trueness:The closeness of agreement between the average value obtained from a large set of test results and an accepted reference value.
- Precision:how close results are to one another



Good Accuracy
Good Precision



Good Precision
Bad Accuracy



Good Accuracy
Bad Precision

Determination of Trueness

- Using Certified Reference Materials
- Using RM or In-house materials
- Using Reference methods
  - Single sample
  - Many samples
- Via Interlaboratory study
- Using injected samples

Determination of Trueness

- Using Certified Reference Materials
  - Six analyses, then calculate the $\bar{x}$
  - $\mu - 2s < \bar{x} < \mu + 2s$

    μ the certified value of CRM

Determination of Trueness

- Using RM or In-house material
  - $\bar{x}$ the average of 10 measurements
  - μ the “true” value of RM
  - If $t_{exp}<t_{theor}$. For v=n-1, no statistically significant differance

$$t_{\exp} = \left| \frac{\bar{X} - \mu}{s} \right| \times \sqrt{n}$$

Determination of Trueness

- Using Reference methods

Single sample

- our own method, N_x, x-bar
- reference method, N_y, y-bar

Condition: $t_{exp} \leq t_{theor.}$ and $F_{exp} < F_{theor.}$

$$t_{\text{exp}} \leq \frac{\left|\bar{x} - \bar{y}\right|}{s_{xy} \times \sqrt{\frac{1}{N_x} + \frac{1}{N_y}}} \qquad s_{xy} = \sqrt{\frac{\sum (\bar{x} - x_i)^2 + \sum (\bar{y} - y_i)^2}{N_x + N_y - 2}} \qquad F_{\text{exp}} = \frac{s_x^2}{s_y^2}, s_x > s_y$$

Determination of Trueness

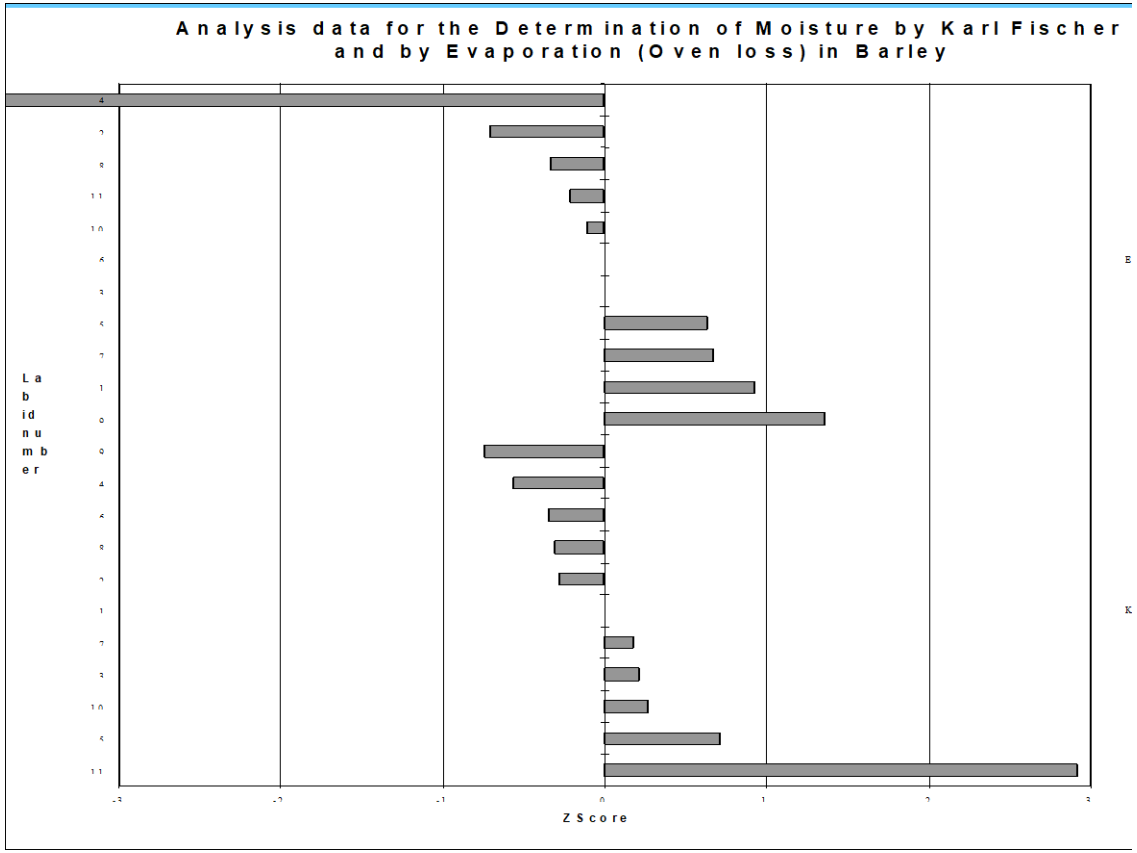
- Many samples
  - Analysed with our own method: $x_1, x_2, .., x_n$
  - Analysed with referee method: $\mu_1, \mu_2, .., \mu_n$

$$t_{\exp} = \frac{\left|\bar{d}\right| \times \sqrt{n}}{s_d}, d = \left|x_i - \mu_i\right|$$

  - Condition: $t_{exp} < t_{theor.}$

Determination of Trueness

- Via Interlaboratory study



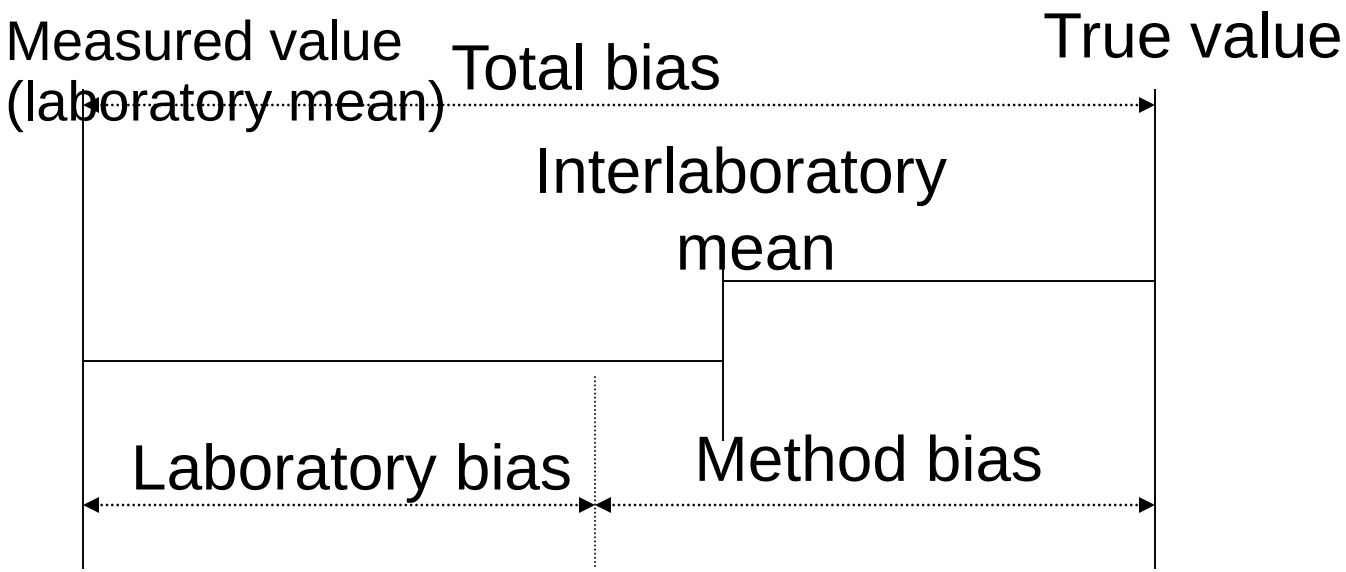
Determination of Trueness

- Using injected samples
  - Injection of a known quantity of a substance in a real sample
  - determination of the recovery

$$\text{Recovery} = \frac{C_{frt} - C_{unf}}{C_{add}} \cdot 100$$

  - C_{frt}: concentration determined in fortified sample
  - C_{unf}: concentration determined in unfortified sample
  - C_{add}: concentration of fortification

Interpretation of Bias



Precision

The closeness of agreement between independent test results obtained under stipulated conditions.

Precision

- Precision under repeatability conditions:
  - Same method on identical test items, in the same laboratory,by the same operator, using the same equipment, within short time intervals
- Precision under reproducibility conditions
  - Same method on identical test items, in different laboratories, with different operators, using different equipment.

Intermediate Precision

- Repeatability conditions and Reproducibility Conditions are extreme cases
- Intermediate cases are most frequent
- Same laboratory - different operators
- Same laboratory - different equipment etc

- ISO 5725

Evaluation of Precision

10 samples for each conc.under r,R, within lab R

- Standard Deviation

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x-\mu)^2}{n}} \qquad \sigma = \sqrt{\frac{\sum_{i=1}^{n}(x-\bar{x})^2}{n-1}} \qquad RSD = \frac{s}{\bar{x}} \times 100$$

  - R=3s (99%confidence, 15 days)

Determination in pairs under r,R, within lab R

- Std. Dev. between two single determinations

$$s = \sqrt{\frac{\sum (a_i - b_i)^2}{2d}}$$

  - a-b, the difference between the values, d, the number of pairs

Precision

- Repeatability limit

$$r = t_{\infty} \times \sqrt{2} \times \sigma_r$$

- Reproducibility limit

$$R = t_{\infty} \times \sqrt{2} \times \sigma_R$$

Sensitivity

The change in the response of a measuring instrument divided by the corresponding change in the stimulus

Ruggedness and Robustness

- Intra-laboratory study to check changes due to environmental and/or operating conditions
  - Usually it is part of method development
  - Deliberate changes in
    - Temperature
    - Reagents (e.g. different batches)
    - Extraction time
    - Composition in the sample
    - etc

The Tools of Validation

- Blanks
  - Reagent blanks
  - Sample blanks
- Samples/test materials
- Fortified materials/solutions
- Spiked materials
- Incurred materials
- Independently characterized materials
- Measurement standards
- Reference materials
- Certified Reference materials
- Statistics
- Replication

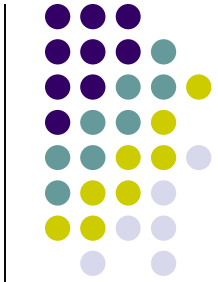
Using Validated Methods

- Make yourself familiar with the new method
- Make necessary preparations before you start working
- Analyze only an optimum number of samples
- Re-validate as appropriate

Validation and Quality Control

- Internal QC
  - QC samples and the use of control charts
    - Warning and action limits
    - QC samples to be within limits
    - Realistic limits on the control chart
  - Various types of blanks to correct the response
  - Replicate analysis to check changes in precision
  - Blind analysis to check precision
  - Standards and chemical calibrants to check the stability of the response

Validation and Quality Control

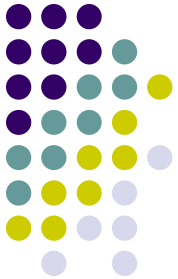


- External QC
  - Proficiency testing
    - Monitor of laboratory performance
    - Highlight reproducibility
    - Monitor of traceability
    - Recognized by accreditation bodies
  - PT results as means of checking QA

Documentation of Validated Methods

- Documentation of the validation procedure
  - Clear and unambiguous implementation
  - Consistency during application
  - Large uncertainty contribution if inadequately documented methods
  - Information to be easily understood by everyone using the method

The Method Documentation Protocol



- Update & Review Summary
- Title
- Scope
- Warning & Safety precautions
- Definitions
- Principle
- Reagents & Materials
- Apparatus and equipment
- Sampling and samples
- Calibration
- Quality Control
- Procedure
- Calculation
- Reporting procedures including expression of results
- Normative references
- Appendix on method validation
- Appendix on measurement uncertainty

Interpretation of Validation Data

- Validation data to give answers and solutions to customer's problems
- Analyst's access to the validation data
- The analytical chemist as a technical advisor
  - Interpretation of the measurement uncertainty of results
  - Legal and forensic contexts
- When reporting the results
  - Either to correct for bias or to acknowledge bias
  - "Not detected" statement to be accompanied by the detection limit
  - Expression of uncertainties

Summary

- The analytical method as a measurement tool
- Validation and revalidation
- Correct use of validation data
- Validation data as a measure of the method performance

Where to Get More Information

- http://www.eurachem.ul.pt
  - The Fitness for purpose of analytical methods, Eurachem Guide
- ISO 5725 –1986 (E), Precision of Test Methods - Determination of repeatability and reproducibility for a standard test method by interlaboratory tests.