Overview

• Importance of planning
• Content of a validation plan
• Experimental designs
• Eurachem guidance
**Importance of planning**

- Method validation is a potentially complex activity
  - Can generate a significant amount of data
- Many decisions to be made
  - Which performance characteristics are important, which materials should be analysed, how many replicates are needed, how should the data be processed, how is ‘fitness-for-purpose’ assessed…
- To ensure the validation study is ‘fit-for-purpose’ all of these issues should be addressed before starting work
- Using a planning template
  - Allows a consistent approach
  - Validation plan can easily be converted to a validation report

**Content of a validation plan**

- Method to be validated
- Status of method and purpose of validation study
- Analytical requirement
- Performance characteristics
- Performance targets
- Summary
- Approval
Content of a validation plan

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Method to be validated – setting the scene

- Validation should start with a documented method
- Method title
- Status of method
  - Standard method (unmodified)
  - Modified standard/published method
  - In-house method
- Purpose of study
  - Full validation
  - Verification
Purpose of validation study

- Why is the validation study being undertaken?
  - Full validation of a method developed in-house
  - Verification of implementation of a published method for which data on performance characteristics are available
  - Validation of change of scope of a method
  - Re-validation following change in operating conditions
  - Re-validation after period of non-use

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Analytical Requirement (1)

- **Analyte**
  - Specify the analyte(s) (e.g. copper, creatinine, hexavalent chromium)

- **Measurand**
  - Quantity intended to be measured
    - Total concentration, amount extracted under specified conditions?
    - Measurement units
    - Required range (e.g. expected concentration range in samples)

<table>
<thead>
<tr>
<th>Analytical requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyte</strong></td>
</tr>
<tr>
<td><strong>Measurand</strong></td>
</tr>
<tr>
<td><strong>Matrix and form</strong></td>
</tr>
<tr>
<td><strong>Purpose of measurement</strong></td>
</tr>
</tbody>
</table>

Analytical Requirement (2)

- **Matrix and form**
  - Sample matrix/matrices, physical form

- **Purpose of measurement**
  - Why are the measurements required?
    - Check compliance with a regulation
    - Monitoring a production process
    - R&D project

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Which performance characteristics need to be evaluated?

<table>
<thead>
<tr>
<th>Performance characteristic</th>
<th>Type of analytical application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Identification</td>
</tr>
<tr>
<td>Selectivity</td>
<td>✓</td>
</tr>
<tr>
<td>Limit of detection</td>
<td></td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td></td>
</tr>
<tr>
<td>Working range/linearity</td>
<td>✓</td>
</tr>
<tr>
<td>Trueness (Bias)</td>
<td>✓</td>
</tr>
<tr>
<td>Precision</td>
<td>✓</td>
</tr>
</tbody>
</table>
Plan for each performance parameter

Specify:
• The performance criteria
• The experiments required
  – Materials to be analysed
  – Number and order of measurements
• Data analysis
  – Appropriate statistics tools
    • Significance tests, analysis of variance, regression
• Assessment of ‘fitness-for-purpose’
  – ‘Rules’ for determining whether performance targets have been met

Experimental designs

• Choosing a suitable experimental design is a key step
  – Maximise the information obtained from an experiment
• May be possible to obtain information on more than one performance characteristic
• Common designs
  – Simple replication
  – Nested
  – Fractional factorial
  – Linear calibration
Simple replication

- Repeated measurements on a single material
- Useful for precision studies
  - Especially repeatability
- Can also be used for evaluating bias
  - If a reference value is available (e.g. material is a CRM)

\[ S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}} \]

Nested design

- Each level of a given factor appears in only a single level of any other factor
- Useful for precision studies
  - Replicate measurements obtained in a short period of time are 'nested' within days or analytical runs
  - Repeatability and intermediate precision can be evaluated
- Analysed using one-way analysis of variance (ANOVA)
Fractional factorial designs

• Factorial design* where carefully chosen combinations of levels have been removed
• Seven factor ‘Plackett-Burman’ design
  – Used in ruggedness studies

*Factorial designs allow the study of multiple parameters at two or more levels. A full factorial design is one in which all combinations of levels are studied.

<table>
<thead>
<tr>
<th>Experimental parameter</th>
<th>Experiment number</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
<tr>
<td>A or a</td>
<td>A A A a a a a a</td>
</tr>
<tr>
<td>B or b</td>
<td>B B b B b B b b</td>
</tr>
<tr>
<td>C or c</td>
<td>C c C c C c c c c</td>
</tr>
<tr>
<td>D or d</td>
<td>D D d d d d D D</td>
</tr>
<tr>
<td>E or e</td>
<td>E e E e e E e E</td>
</tr>
<tr>
<td>F or f</td>
<td>F f F f f f f F</td>
</tr>
<tr>
<td>G or g</td>
<td>G g G g G G G g</td>
</tr>
<tr>
<td>Observed result</td>
<td>s t u v w x y z</td>
</tr>
</tbody>
</table>

Content of a validation plan

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Performance targets

- Performance targets need to be established to assess fitness-for-purpose of the method
- Target values can be:
  - Defined in standards/regulations
  - Specified by the customer
  - Stated in a standard published method (can you match the stated performance?)
  - Based on performance of similar procedures that are known to be fit-for-purpose
  - Defined as the current state-of-the-art (what is the method capable of)?

Content of a validation plan

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- Performance targets
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Summary and approval

- Validation plan should be approved
- After study is complete
  - Provide a summary of values and other information obtained for each performance characteristic
  - Final statement on whether the aims of the study have been met and whether method is fit-for-purpose
  - Final sign-off of the validation report

Eurachem guidance

- Available from www.eurachem.org
- The Fitness for Purpose of Analytical Methods
- Supplement: Planning and Reporting Method Validation Studies
Summary

• Validation should be a planned and documented activity
• For each performance characteristic specify
  – Materials, number of measurements, order, data analysis, performance criteria
• Planning template recommended
  – Consistent approach
  – Easily converted to a validation report
• Both plan and final report should be signed off

Any questions?