Develop a Quality by Design (QbD) Approach for Analytical Method Development

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SESSION 1 – ANALYTICAL PROCEDURES AND METHOD VALIDATION
Jane Weitzel has been working in analytical chemistry for over 35 years for mining and pharmaceutical companies with the last 5 years at the director/associate director level. She is currently a consultant, auditor, and trainer. Jane has applied Quality Systems and statistical techniques, including the estimation and use of measurement uncertainty, in a wide variety of technical and scientific businesses. She has obtained the American Society for Quality Certification for both Quality Engineer and Quality Manager.

In 2014 she was pointed to the Chinese National Drug Reference Standards Committee and attended their inaugural meeting in Beijing.

For the 2015 – 2020 cycle, Jane is a member of the USP Statistics Expert Committee and Expert Panel on Method Validation and Verification.
Develop a Quality by Design (QbD) Approach for Analytical Method Development

Stage 1 in the Lifecycle of an Analytical Procedure - Method Development

Using Design of Experiments and ANOVA

Estimating and Using Measurement Uncertainty
Based on Approach

http://www.friesenpress.com/bookstore/title/119734000004601536
Talks at Conference

Session 1 – Analytical Procedures and Method Validation
Develop a Quality by Design (QbD) Approach for Analytical Method Development

Session 6 – Analytical Procedures and Method Validation
Method Validation Characteristics through Statistical Analysis Approaches

Session 8 - Investigating Laboratory OOS Test Results
Manage Risk and Improve Efficiency in the Laboratory
Lifecycle of Analytical Procedure

Stage 1
Design Development & Understanding

Stage 2
Performance Qualification

Stage 3
Continued Performance Verification

Session 1 – Analytical Procedures and Method Validation
Develop a Quality by Design (QbD) Approach for Analytical Method Development

Session 6 – Analytical Procedures and Method Validation
Method Validation Characteristics through Statistical Analysis Approaches

Session 8 - Investigating Laboratory OOS Test Results
Manage Risk and Improve Efficiency in the Laboratory
Justification for Approach

WHY?
The FDA Guidance discusses the role of the analytical procedure in the lifecycle of the product to meet its requirements:

- Good science
- Metrology.
Sound Science

- Metrological approach to measurements
  - Measurement uncertainty
  - Target measurement uncertainty
  - Completely characterises the variability

http://www.fda.gov/ScienceResearch/FieldScience/LaboratoryManual/ucm171878.htm

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Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures …

CFR has always required use of sound science: Decision rules, measurement uncertainty, risk and probability have been used in many scientific areas for many years.
(d) Acceptance criteria for the sampling and testing conducted by the quality control unit shall be adequate to assure that batches of drug products meet each appropriate specification and appropriate statistical quality control criteria as a condition for their approval and release. The statistical quality control criteria shall include appropriate acceptance levels and/or appropriate rejection levels.
Develop a Quality by Design (QbD) Approach for Analytical Method Development

Stage 1 in the Lifecycle of an Analytical Procedure - Method Development

Using Design of Experiments and ANOVA

Estimating and Using Measurement Uncertainty
LIFE CYCLE MANAGEMENT OF ANALYTICAL PROCEDURES:
METHOD DEVELOPMENT, PROCEDURE PERFORMANCE
QUALIFICATION, AND PROCEDURE PERFORMANCE VERIFICATION

ABSTRACT
In this Stimuli article, the USP Validation and Verification Expert Panel discusses the modern concept of lifecycle management, which is based on process validation and described in ICH guidelines Q6, Q7, and Q8. The approach is applied to analytical procedures. The Expert Panel proposes that the traditional approaches to validation, transfer, and verification should be integrated into the lifecycle management process rather than being viewed as separate entities. As a starting point or “predefined objective” according to ICH Q8, the requirements for measurement of critical quality attributes are established in the Analytical Target Profile. In alignment with process validation, three stages are proposed: Procedure Design (development and understanding), Procedure Performance Qualification, and Continued Procedure Performance Verification.

INTRODUCTION
Any analytical procedure must be shown to be fit for its intended purpose before use. [Note—The term analytical procedure used in this Stimuli article is interchangeable with the term “lifecycle management.”] The term “lifecycle management” is commonly used in industry and includes steps such as sample preparation, analytical technique, calibration, and definition of the reportable result (1). The usual process of demonstrating this suitability in food and drug analytical laboratories takes place by way of a documented validation study and, if required, a verification or transfer process to demonstrate the procedure performs appropriately in the laboratory in which it will be used. The United States Pharmacopeial Convention (USP) has been a strong advocate of this approach. General chapter Validation of Analytical Procedures (1221), which was first published in USP XXII (1999), served as the foundation for the development of the ICH Q7 Guidance on Validation of Analytical Procedures. More recently, USP has furthered this topic with the publication of general chapters...
Lifecycle Management of an Analytical Procedure

- The Stimuli article discusses how the modern concept of lifecycle can be applied to analytical procedures.
- In December 8 & 9, 2014, the approach was further explained and the required statistical tools were presented at a USP workshop.
- Series of articles will be published to further explain these concepts with worked examples.
Analytical Target Profile (ATP)

- A predefined objective that states the performance requirements for the analytical procedure
- The output of the procedure is a reportable result.
- The reportable result must be fit for its purpose.
Wording for ATP

- Assay

The procedure must be able to quantify the analyte in presence of \((X, Y, Z)\) over a range of \(A\%\) to \(B\%\) of the nominal concentration with an accuracy and uncertainty so that the reportable result falls within \(\pm C\%\) of the true value with at least \(P\%\) probability.

- The variables in orange are specific for each reportable result.
LET’S LOOK AT THESE TERMS
Accuracy and uncertainty are defined

- Accuracy is represented by the bias.
- The uncertainty is the Target measurement uncertainty
Improving Trueness

Improving Precision

Accuracy contains both bias and precision components

Bias is the total systematic error

Uncertainty includes all random effects (including the uncertainty of the bias)

How uncertainty relates to accuracy and precision
2.26 (3.9) measurement uncertainty
uncertainty of measurement
uncertainty non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used

(Standard uncertainty is analogous to a standard deviation. It is abbreviated as “u”)
Target Measurement Uncertainty

- 2.34
- target measurement uncertainty
- target uncertainty
- Measurement uncertainty specified as an upper limit and decided on the basis of the intended use of measurement results

The target measurement uncertainty becomes part of the analytical target profile.

The TMU defines the acceptance criteria for the method.

Remember, the uncertainty includes all random effects (including the uncertainty of the bias).
Terminology

- Explains the terminology

- http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/TechnicalBriefs.asp
Probability

LANGUAGE FOR QBD
Basic Concept - Probability

- A basic concept for this approach to analytical procedures is probability.
- Common usage definition
  - The chance that something will happen.

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Statistical usage

- **probability**
  - a real number in the scale 0 to 1 attached to a random event
  - NOTE It can be related to a long-run relative frequency of occurrence or to a degree of belief that an event will occur.
  - For a high degree of belief, the probability is near 1.
  - [ISO 3534-1:1993, definition 1.1]
Probability

- In the lifecycle approach which uses measurement uncertainty, probability is used to express the fitness for use.
- Allows for clear, unambiguous, quantitative communication.
Example - Do you release the lot?

- A lot of drug substance is ready to be released.
  - Specification is 90.0 to 110.0%
  - Value is 93.7%.

- Do you release the lot?
Example – Release of a Lot

**Common Usage**
- The lot can be released because the chance of it being Out Of Specification, OOS, is low.
- Potency is 93.7%

**Statistical Usage**
- The lot can be released because the probability of the potency being OOS is < 0.3%.
- Potency is 93.7% ± 3.0 % with a coverage factor of 3 for a 99.7% level of confidence
Concentration

<table>
<thead>
<tr>
<th>Upper Limit</th>
<th>Lower Limit</th>
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<tbody>
<tr>
<td>90</td>
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<td>106</td>
<td>108</td>
</tr>
<tr>
<td>108</td>
<td>110</td>
</tr>
</tbody>
</table>

Normal Distribution

Probability

93.7

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Nominal Concentration (Central Value) 93.7
Target Measurement Uncertainty (Standard Deviation) 1.00
Enter the largest standard deviation which results in the acceptable "Total outside limits".
Lower Limit 90.0
Upper Limit 110.0

% below Lower Limit 0.011%
% above upper limit 0.00%
Total outside limits 0.0%

Concentration

Lower Limit
Upper Limit

Uses EXCEL

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Normal Distribution Curve

Y axis can be thought of as the likelihood of obtaining a value

X axis can be concentration

σ is the standard deviation

μ

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Normal Distribution Curve

Y axis can be thought of as the likelihood of obtaining a value

X axis can be concentration

σ is the standard deviation

area under the curve between two distinct points defines the probability for that interval

µ

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% of population between z values

Percentage of Results Between z Factors for a Normal Distribution

For example, the probability for interval between z=1 and z=-1 is 68%
There are different types of statistical tests for various purposes.

They are often based on the concept of probability. How likely is something???

In our example, the likelihood is < 0.3%.
Established Concepts

- These presentations will explain what these concepts are and how to use them.
- They are based on established statistical and metrological concepts that are used by many industries:
  - Food
  - Nuclear
  - Traffic Laws, etc.
The USER Develops Decision Rule

- For an example, consider the case for a brand new measurement

- The best source for the prescription of the decision rule is the person/organization that will use the output of the analytical procedure
  - Can be one person (the expert) or a group of people (clinical studies, production, stability)
  - The group can include management (financial risks)
  - Called Decision Makers in ICH Q8
Intended Use can be Linked to Clinical Requirement
From where does information come?

- Could be Arbitrary
  - Assign the values based on expert judgement, past experience, regulatory requirement/request
  - The problem is that this assignment of values is not based on the intended use.
Information can come from aQbD

- Analytical Quality by Design (aQbD) can use tools based on sound science, good metrological practices and statistics to provide the information for the ATP.
  - Decision rules
  - Measurement uncertainty (u)
  - Target measurement uncertainty (TMU)
Cyclical Process – May have to revisit the Decision Rule

Decision Rule

Target Measurement Uncertainty

ATP

Analytical Procedure Performance Characteristics

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Decision Rules – References

Guidelines for Decision Rules: Considering Measurement Uncertainty in Determining Conformance to Specifications

EURACHEM / CITAC Guide
Use of uncertainty information in compliance assessment
First Edition 2007

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A documented rule ... that describes how measurement uncertainty will be allocated with regard to accepting or rejecting a product according to its specification and the result of a measurement.

- ASME B89.7.3.1-2001 (reaffirmed 2006)
Decision Rule – Acceptance or Rejection

Decision rules give a prescription for the acceptance or rejection of a product based on the measurement result, its uncertainty and the specification limit or limits, taking into account the acceptable level of the probability of making a wrong decision.
Why Use Decision Rules

- Decision rules clearly state the intended use of the procedure.
- Risk and probability are used to develop the decision rule.
  - This means there is a defined process to define the intended use of the procedure.
  - Risk and Probability are consistent with QbD.
- A guard band can be created using the uncertainty.
Decision Rule

To decide whether a result indicates compliance or non-compliance with a specification, it is necessary to take into account the measurement uncertainty.

1. Result is above the limit. Limit is below expanded uncertainty.
2. Result above the limit. Limit is within the expanded uncertainty.
3. Result is below the limit. Limit is within the expanded uncertainty.
4. Result is below the limit. Limit is above expanded uncertainty.
Decision Rules Require 4 Components

- Decision rules give a prescription for the acceptance or rejection of a product based on
  1. the measurement result,
  2. its uncertainty and
  3. the specification limit or limits,
  4. taking into account the acceptable level of the probability of making a wrong decision.
Types of Decision Rules

- There are several types of decision rules
- We will discuss
  - a simple decision rule
  - Guard bands
**Simple Acceptance and Rejection**

- Product conformance is verified if the measurement result lies in the specification zone and rejection is verified otherwise.

(This decision rule could be applied to most monograph specifications.)
50% Probability

- If a result is obtained at or near limit the probability of true value being outside the limit is 50%
Add guard band
(sometimes called internal release limit)

The guard band is determined using the acceptable probability of making a wrong decision.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>% below Lower Limit</td>
<td>2.28%</td>
<td></td>
</tr>
<tr>
<td>% above upper limit</td>
<td>0.00%</td>
<td></td>
</tr>
<tr>
<td>Total outside limits</td>
<td>2.3%</td>
<td></td>
</tr>
</tbody>
</table>
For the Statistical Approach

- Decision rule includes:
- … taking into account the acceptable level of the probability of making a wrong decision.
- The statistical approaches that will be discussed next require a probability
SELECT A TECHNOLOGY OR METHOD
Based on Reportable Result Requirements

Based on TMU & probability

- Select suitable technology
- E.g. use ELISA screening method or ICP-MS-MS
Use your knowledge to select the technique and explain the choice using uncertainty and probability

- E.g.
  - Elisa screening kit has uncertainty of 14%
  - HPLC has uncertainty of 1%
ELISA Screening

Nominal Concentration (Central Value): 100.0
Target Measurement Uncertainty (Standard Deviation): 14.00
Lower Limit: 0.0
Upper Limit: 200.0

% below Lower Limit: 0.00%
% above upper limit: 0.00%
Total outside limits: 0.00%
Nominal Concentration (Central Value) 100.0
Target Measurement Uncertainty (Standard Deviation) 1.00
Enter the largest standard deviation which results in the acceptable "Total outside limits".

Lower Limit 90.0
Upper Limit 110.0

% below Lower Limit 0.00%
% above upper limit 0.00%
Total outside limits 0.0%

Concentration
Procedure Design - Robustness
DOE

- DOE is a useful, efficient tool for procedure validation
- Well planned experiments
- Hint: finalize and clearly define how the data will be assessed/analyzed as part of the protocol. Include actual formulas, charts, tables, etc.
  - Doing so makes for a well thought out, easily understood protocol.
  - It also makes writing the report faster and easier.
  - You are less likely to have to repeat experiments.
    - Penny wise is often pound foolish.
Ruggedness DOE - Definitions

- USP <1225> Validation of Compendial Procedures

- Ruggedness - Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory.

- Robustness - The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in procedural parameters listed in the procedure documentation ...
DOE Presented Here and in Workbook

- It is an “incomplete factorial design”
- Also known as
  - AOAC Ruggedness Test
  - Youden

- There are many published examples that you can use as guides.
  - Determine which factors were varied.
Robustness test

- Often done during the design stage of the procedure
  - If so, the test can be referenced and summarized in the final report of the analytical procedure qualification
- Allows much information to be learned from a relatively small number of tests
# Run Design

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<th>2</th>
<th>3</th>
<th>4</th>
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<td>B</td>
<td>C</td>
<td>D</td>
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<td>Result</td>
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<td>u</td>
<td>v</td>
<td>w</td>
<td>x</td>
<td>y</td>
<td>z</td>
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Calculations for Factor A

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<td>u</td>
<td>v</td>
<td>w</td>
<td>x</td>
<td>y</td>
<td>z</td>
</tr>
</tbody>
</table>

- Effect of altering level is calculated:
- E.g. Factor A \( \frac{(s+t+u+v)}{4} - \frac{(w+x+y+z)}{4} \)
Comparison of Factors

- Compare the difference for factors.
- Any difference that is substantially larger than the others is significant.
- If no factors are significant, take the standard deviation of all experiments as the robustness standard deviation.
- Even if a factor is significant, if the overall standard deviation is less than the TMU, the procedure is still fit for use.
  - The factor can be left as is.

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What to do with Significant Factor?

- When a factor is found to be significant there are a few options
  - Control the factor
  - Change the factor slightly
  - Worst case, go back to procedure development
The overall standard deviation from all the tests can be a good estimate of precision (uncertainty).

- Must know what was varied during and between experiments (beside the factors), eg.
  - Different instruments?
  - Different days?
  - Different calibration solutions?
Example of Factors Selected for HPLC

Factors

- extraction heating time,
- extraction temperature,
- HCl concentration,
- extraction volume,
- column temperature,
- flow rate, and
- test sample weight
Examples in Literature

Ruggedness testing of the official method for rot fragments in...
www.ncbi.nlm.nih.gov/pubmed/4055635
by GE Russell - 1985
The official AOAC method for rot fragments in comminuted tomato products (44.224)
has been revised on the basis of Youden's ruggedness testing procedures...

Determination of Flavonol Aglycones in Ginkgo biloba Dietary...
by D Gray - 2005 - Cited by 9 - Related articles
Nov 25, 2008 - ... A Youden ruggedness trial testing 7 factors with the potential to
affect quantitative results showed that 2 factors (volume hydrolyzed and test...
Design of experiments is a discipline in itself.

More information on DOE and the robustness test presented above can be found in the

- AOAC book *Use of Statistics to Develop and Evaluate Analytical Methods*
- *Application of ISO/IEC 17025 Technical Requirements in Industrial Laboratories; Method Validation*, M. L. Jane Weitzel and Wesley M. Johnson
New Proposed USP General Chapter 1210 Statistical Tools for Procedure Validation

- New proposed USP General Chapter <1210> Statistical Tools for Method Validation.
- This new proposed chapter, which was published in PF 40(5) [Sept-Oct 2014], will be a statistical companion chapter for USP General Chapter <1225> Validation of Compendial Procedures.

Watch for this!

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Analysis of Variance

ANOVA
Useful

- ANOVA is a powerful and useful statistical tool that is underused
- Often the analytical procedure contains the data for an ANOVA assessment, but it is not done
- It allows the estimation of relative sizes of variances
  - Can quantify the size of uncertainty components
## Typical Experiment for Accuracy

- **Look at data**
- **Could calculate average and standard deviation**
- **Could do t test**
  - Only tell if 2 spikes were different
  - Only tells if they are different, no probability
- **“It looks good. ??”**

### “Accuracy Experiment”
3 spikes done on same day

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Spike 1</th>
<th>Spike 2</th>
<th>Spike 3</th>
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<tbody>
<tr>
<td>1</td>
<td>99.53</td>
<td>102.30</td>
<td>100.78</td>
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<tr>
<td>2</td>
<td>101.88</td>
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<td>101.88</td>
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<table>
<thead>
<tr>
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<th>Average</th>
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</thead>
<tbody>
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<td>Spike 1</td>
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<tr>
<td>Spike 2</td>
<td>100.72</td>
<td>2.18</td>
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<tr>
<td>Spike 3</td>
<td>101.25</td>
<td>1.58</td>
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## ANOVA One-way EXCEL Print Out

### Anova: Single Factor

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<tr>
<th>Groups</th>
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<th>Sum</th>
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<th>Variance</th>
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</thead>
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<td>99.90167</td>
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<td>Spike 3</td>
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<td>607.48</td>
<td>101.2467</td>
<td>2.502467</td>
</tr>
</tbody>
</table>

### ANOVA

<table>
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<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5.5083</td>
<td>2</td>
<td>2.75415</td>
<td>0.563798</td>
<td>0.580647</td>
<td>3.68232</td>
</tr>
<tr>
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<td>15</td>
<td>4.884997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>78.78325</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Calculate Precisions

<table>
<thead>
<tr>
<th>Precision</th>
<th>Symbol</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>repeatability</td>
<td>( s_r )</td>
<td>( \sqrt{MS \text{ Within Groups}} )</td>
</tr>
<tr>
<td>Between Group Standard Deviation</td>
<td>( s_{BG} )</td>
<td>( \sqrt{(MS \text{ Between Groups} - MS \text{ Within Groups})/\text{Count}} )</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>( s_{IP} )</td>
<td>( \sqrt{S_r^2 + S_{BG}^2} )</td>
</tr>
</tbody>
</table>

### ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

### Precisions

<table>
<thead>
<tr>
<th>Precisions</th>
<th>Symbol</th>
<th>Value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>( s_r )</td>
<td></td>
<td>2.210203</td>
<td></td>
</tr>
<tr>
<td>( s_{BG} )</td>
<td>#NUM!</td>
<td>The MS's do not differ significantly. Both estimate repeatability.</td>
<td></td>
</tr>
<tr>
<td>( s_{IP} )</td>
<td>#NUM!</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Repeatability

Anova: Single Factor

## SUMMARY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
<th>Std Dev</th>
<th>s&lt;sub&gt;r&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike 1</td>
<td>6</td>
<td>599.41</td>
<td>99.90167</td>
<td>7.378857</td>
<td>2.716405</td>
<td>2.210203</td>
</tr>
<tr>
<td>Spike 2</td>
<td>6</td>
<td>604.3</td>
<td>100.7167</td>
<td>4.773667</td>
<td>2.184872</td>
<td></td>
</tr>
<tr>
<td>Spike 3</td>
<td>6</td>
<td>607.48</td>
<td>101.2467</td>
<td>2.502467</td>
<td>1.581919</td>
<td></td>
</tr>
</tbody>
</table>

## ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
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<th>MS</th>
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<td>Total</td>
<td>78.78325</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Precisions: $s_r = 2.210203$
Between Groups & Intermediate Precision (IP)

<table>
<thead>
<tr>
<th>Precision</th>
<th>Symbol</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>repeatability</td>
<td>$s_r$</td>
<td>$\sqrt{MS \text{ Within Groups}}$</td>
</tr>
<tr>
<td>Between Group Standard Deviation</td>
<td>$s_{BG}$</td>
<td>$\sqrt{\left(\frac{MS \text{ Between Groups} - MS \text{ Within Groups}}{\text{Count}}\right)}$</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>$s_{IP}$</td>
<td>$\sqrt{s_r^2 + s_{BG}^2}$</td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5.5083</td>
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<td>2.75415</td>
<td>0.563798</td>
<td>0.580647</td>
<td>3.68232</td>
</tr>
<tr>
<td>Within Groups</td>
<td>73.27495</td>
<td>15</td>
<td>4.884997</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Precisions**

- $s_r$: 2.210203
- $s_{BG}$: #NUM!
- $s_{IP}$: #NUM!

The MS's do not differ significantly. Both estimate repeatability.

The between group difference is not significant.
Set $s_{BG}$ to 0.
Not unexpected given 3 spikes done on same day.
<table>
<thead>
<tr>
<th>Replicate</th>
<th>Condition 1</th>
<th>Condition 2</th>
<th>Condition 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.03</td>
<td>103.30</td>
<td>103.78</td>
</tr>
<tr>
<td>2</td>
<td>101.38</td>
<td>102.40</td>
<td>107.00</td>
</tr>
<tr>
<td>3</td>
<td>100.79</td>
<td>97.60</td>
<td>103.26</td>
</tr>
<tr>
<td>4</td>
<td>101.38</td>
<td>101.70</td>
<td>102.57</td>
</tr>
<tr>
<td>5</td>
<td>94.21</td>
<td>103.70</td>
<td>103.78</td>
</tr>
<tr>
<td>6</td>
<td>99.62</td>
<td>101.60</td>
<td>105.09</td>
</tr>
</tbody>
</table>

Average | 99.40 | 101.72 | 104.25 |
Std Dev  | 2.72  | 2.18   | 1.58   |

- What could condition be?
  - Condition could be:
    - day
    - Instrument
    - reagents
- Look at average & standard deviation

mljweitzel@msn.com
## ANOVA

### Anova: Single Factor

#### SUMMARY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition 1</td>
<td>6</td>
<td>596.41</td>
<td>99.40167</td>
<td>7.378857</td>
</tr>
<tr>
<td>Condition 2</td>
<td>6</td>
<td>610.3</td>
<td>101.7167</td>
<td>4.773667</td>
</tr>
<tr>
<td>Condition 3</td>
<td>6</td>
<td>625.48</td>
<td>104.2467</td>
<td>2.502467</td>
</tr>
</tbody>
</table>

#### ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>70.4683</td>
<td>2</td>
<td>35.23415</td>
<td>7.212728</td>
<td>0.006386</td>
<td>3.68232</td>
</tr>
<tr>
<td>Within Groups</td>
<td>73.27495</td>
<td>15</td>
<td>4.884997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143.7433</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Precisions

<table>
<thead>
<tr>
<th>Precisions</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s_r$</td>
<td>2.210203</td>
</tr>
<tr>
<td>$s_{BG}$</td>
<td>2.249043</td>
</tr>
<tr>
<td>$s_{IP}$</td>
<td>3.153282</td>
</tr>
</tbody>
</table>
Uncertainty Components

- Both repeatability and between condition will impact the variability – the uncertainty
- The conditions are critical variables and will need some form of operational control

<table>
<thead>
<tr>
<th>Precisions</th>
<th>$s_r$</th>
<th>2.21</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s_{BG}$</td>
<td></td>
<td>2.25</td>
</tr>
<tr>
<td>$s_{IP}$</td>
<td></td>
<td>3.15</td>
</tr>
</tbody>
</table>
If replication is needed (the test portion is analyzed more than a singlicate), where are the replicates placed?

Between runs is best

<table>
<thead>
<tr>
<th>Precisions</th>
<th>$s_r$</th>
<th>2.21</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s_{BG}$</td>
<td></td>
<td>2.25</td>
</tr>
<tr>
<td>$s_{IP}$</td>
<td></td>
<td>3.15</td>
</tr>
<tr>
<td>Replicate</td>
<td>Condition 1</td>
<td>Condition 2</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>89.53</td>
<td>103.30</td>
</tr>
<tr>
<td>2</td>
<td>91.88</td>
<td>102.40</td>
</tr>
<tr>
<td>3</td>
<td>91.29</td>
<td>97.60</td>
</tr>
<tr>
<td>4</td>
<td>91.88</td>
<td>101.70</td>
</tr>
<tr>
<td>5</td>
<td>84.71</td>
<td>103.70</td>
</tr>
<tr>
<td>6</td>
<td>90.12</td>
<td>101.60</td>
</tr>
<tr>
<td>Average</td>
<td>89.90</td>
<td>101.72</td>
</tr>
<tr>
<td>Std Dev</td>
<td>2.72</td>
<td>2.18</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1141.048</td>
<td>2</td>
<td>570.5242</td>
<td>116.7911</td>
<td>7.16E-10</td>
<td>3.68232</td>
</tr>
<tr>
<td>Within Groups</td>
<td>73.27495</td>
<td>15</td>
<td>4.884997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1214.323</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Precisions

- $s_r$: 2.21
- $s_{BG}$: 9.71
- $s_{IP}$: 9.96

The repeatability is not significant.

The between conditions is the critical variable and needs operational control.

How should replicates be placed?
Use MU and Probability to describe performance

<table>
<thead>
<tr>
<th>Nominal Concentration (Central Value)</th>
<th>100.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Measurement Uncertainty (Standard Deviation)</td>
<td>9.96</td>
</tr>
<tr>
<td>Lower Limit</td>
<td>75.0</td>
</tr>
<tr>
<td>Upper Limit</td>
<td>125.0</td>
</tr>
</tbody>
</table>

Enter the largest standard deviation which results in the acceptable "Total outside limits".

<table>
<thead>
<tr>
<th>% below Lower Limit</th>
<th>0.60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% above upper limit</td>
<td>0.60%</td>
</tr>
<tr>
<td>Total outside limits</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

In this case the acceptable probability of making a wrong decision is 5%.
The test item does not need to be centered in specification

Nominal Concentration (Central Value): 92.0
Target Measurement Uncertainty (Standard Deviation): 9.96
Lower Limit: 75.0
Upper Limit: 125.0

% below Lower Limit: 4.39%
% above upper limit: 0.05%
Total outside limits: 4.4%
Add 20% to MU to compensate for its variability

Nominal Concentration (Central Value) 100.0
Target Measurement Uncertainty (Standard Deviation) 11.95
Lower Limit 75.0
Upper Limit 125.0

Enter the largest standard deviation which results in the acceptable "Total outside limits".

% below Lower Limit 1.82%
% above upper limit 1.82%
Total outside limits 3.6%

Multiply the MU by 20%.
1.2 * 9.96 = 11.95

New Eurachem Guide on Setting and Using Target Uncertainty in Chemical Measurements
Understand & Communicate

- Using DOE correctly and effectively allows the identification of critical variables (significant uncertainty components) during analytical procedure design and qualification.
- Adequate operational controls can be implemented.
- The intermediate precision is an initial estimate of uncertainty (for those conditions varied).
  - It can be compared to the target uncertainty.
The performance of the procedure can be communicated quantitatively using probability

- With the intermediate precision as the initial estimate of the uncertainty, the probability of making a wrong decision is 1.2%, less than the 5% required by the decision rule.
HOW TO ESTIMATE MU
How to Estimate Uncertainty

EURACHEM CITAC Guide - Quantifying Uncertainty In Analytical Measurements and VAM Project 3.2.1 Development And Harmonization Of Measurement Uncertainty Principals - Part (d): Protocol for uncertainty evaluation from validation data

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## Process

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
</table>
| **1** | • Identify the measurand  
      • (set up the final concentration calculation equation) |
| **2** | • List the steps in the analytical process |
| **3** | • Identify potential sources of random variability in each step – uncertainty components |
| **4** | • Design a process that permits an estimate of each source of random variability or of a group of sources or look for the data |
| **5** | • Combine the different estimates of random variability to get the overall uncertainty estimate |
2

- List the steps in the analytical process

- Sampling, sub-sampling
- Comminution
- Weighing a test portion
- Dissolving sample (not a dissolution test)
- Volumetric manipulation
- Calibration of measurement
- Instrumental measurement
- Data processing
• Identify potential sources of random variability in each step – uncertainty components

- Use experience
- Brainstorm
- Fishbone
- Equation
- SOP

\[ \sqrt{(s_R^2 + s_r^2)} \]
Fish Bone – Eurachem Guide Quantifying Uncertainty in Analytical Measurement

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Intermediate Precision

- Well designed Design of Experiments activities conducted during analytical procedure development and qualification will yield a good initial estimate of measurement uncertainty.
  - It will not include the long term uncertainty component, but that should be assessed and confirmed during the continued verification stage of the lifecycle.
• Design a process that permits an estimate of each source of random variability or of a group of sources or look for the data.
Method Validation Studies

- These give short term (within-run) uncertainty information over the concentration range validated.

- The use of blanks and reference materials run under replicability conditions provides uncertainty information for those conditions with “no” sampling uncertainty.
Replicate Data to Cover off Sources of Variability

- As part of method validation studies, there are several measures that are repeated.
- As part of routine runs, data has been collected that has repeated measurements while some parameters have varied.
- Combine data
- Can organize using a table

- Combine the different estimates of random variability to get the overall uncertainty estimate
Example from REAL data

The estimation and use of measurement uncertainty for a drug substance test procedure validated according to USP <1225>

M. L. Jane Weitzel
Conclusion

- The decision rule, ATP, and TMU clearly define the purpose of the reportable result and direct the analytical procedure design and development.

- The DOE and ANOVA are effective experimental processes and can provide an initial estimate of the uncertainty.

- The capability of the analytical procedure can be communicated clearly, using probability.
Disclaimer

- This presentation reflects the speaker’s perspective on this topic and does not necessarily represent the views of USP or any other organization.