Method Validation Characteristics through Statistical Analysis Approaches

Jane Weitzel
mljweitzel@msn.com
SESSION 6 – ANALYTICAL PROCEDURES AND METHOD VALIDATION

1:00 to 2:30
Wednesday, Dec. 9
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.
Method Validation Characteristics through Statistical Analysis Approaches

- Fitness for Purpose, the key to analytical procedure qualification characteristics
- How to Set Target Measurement Uncertainty
- Translating TMU to Requirements for Precision, Linearity, Accuracy, etc.
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

mljweitzel@msn.com
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

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Manage Risk and Improve Efficiency in the Laboratory
The FDA Guidance discusses the role of the analytical procedure in the lifecycle of the product to meet its requirements:

- Good science
- Metrology.

The ultimate factor is:

For what will the reportable result be used?

The acceptance criteria for the analytical procedure qualification are driven by the purpose for the result.
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 Rules – References

GUIDELINES FOR DECISION RULES: CONSIDERING MEASUREMENT UNCERTAINTY IN DETERMINING CONFORMANCE TO SPECIFICATIONS

AN AMERICAN NATIONAL STANDARD

EURACHEM / CITAC Guide

Use of uncertainty information in compliance assessment

First Edition 2007

mljweitzel@msn.com
The USER Develops the 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
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.
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 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.

How much overlap is acceptable? That is the acceptable probability of making a wrong decision.
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

You know where you are going

- Purpose is defined and understood
- Acceptable probability of making a wrong decision is defined
- Target Measurement Uncertainty is known
- You have the information to set the acceptance criteria for the analytical procedure qualification
More Information

CALCULATE TMU
Normal Distribution

Nominal Concentration (Central Value): 100.0
Target Measurement Uncertainty (Standard Deviation): 2.00

<table>
<thead>
<tr>
<th>Value</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td></td>
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<tr>
<td>89</td>
<td></td>
</tr>
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<td>95</td>
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<td>97</td>
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<td>99</td>
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<tr>
<td>101</td>
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<tr>
<td>103</td>
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<td>105</td>
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<td>107</td>
<td></td>
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<td>109</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td></td>
</tr>
<tr>
<td>113</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

Lower Limit: 90.0
Upper Limit: 110.0

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

---

Diagram showing a normal distribution curve with concentration values ranging from 85 to 115.
50% probability at the limits

Nominal Concentration (Central Value) 90.0
Target Measurement Uncertainty (Standard Deviation) 2.00
Lower Limit 90.0
Upper Limit 110.0

% below Lower Limit 50.00%
% above upper limit 0.00%
Total outside limits 50.0%

Concentration

Lower Limit

Upper Limit
You Can Set Guard Bands

- If you do not like the 50% probability, in your laboratory, you can do risk analysis and decide on acceptable probability of failure.
  - You could set a guard band.

- Then you can use that probability of failure to calculate the target measurement uncertainty.
The decision makers have established the acceptable probability of making a wrong decision is 5%
Nominal Concentration (Central Value) 93.3
Target Measurement Uncertainty (Standard Deviation) 2.00

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

Lower Limit 90.0
Upper Limit 110.0

% below Lower Limit 4.95%  % above upper limit 0.00%  Total outside limits 4.9%

93.3 & 106.7 Guard Bands

Guard Bands

Concentration
Calculating TMU

- Concentration range of guard band = $g$

\[
TMU = \frac{g}{z_P}
\]

- $z_P$ is the $z$ factor for the normal standard variable for probability, $P$
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.
Cyclical Process – May have to revisit the Decision Rule

- Target Measurement Uncertainty
- Decision Rule
- ATP
- Analytical Procedure Performance Characteristics
Set Requirements

PERFORMANCE CHARACTERISTICS
TMU

- TMU is an “overreaching” acceptance criterion for the analytical procedure qualification
- The source of the uncertainty is not important as long as the combined uncertainty is acceptable
Bias Precision Tradeoff

- If the analytical procedure has a bias, that impacts the uncertainty
  - Should eliminate the bias
  - If that is not possible, adjust the TMU so decision rule requirement for probability is still met
No Bias

Nominal Concentration (Central Value) 100.0
Target Measurement Uncertainty (Standard Deviation) 1.00
Lower Limit 98.0
Upper Limit 102.0

% below Lower Limit 2.28%
% above upper limit 2.28%
Total outside limits 4.6%
Bias -1

Nominal Concentration (Central Value) 99.0
Target Measurement Uncertainty (Standard Deviation) 1.00
Enter the largest standard deviation which results in the acceptable "Total outside limits".
Lower Limit 98.0
Upper Limit 102.0

% below Lower Limit 15.87%
% above upper limit 0.13%
Total outside limits 16.0%

96 97 98 99 100 101 102 103 104
Concentration
Adjust TMU to 0.6 to meet 5%
Correct for Bias

- If you correct for the bias, there is no need to adjust the TMU
- Must remember to include the uncertainty from the correction in the combined uncertainty
Acceptance Criteria for Qualification

- Combination of Bias and Uncertainty meets target measurement uncertainty and meets the decision rule requirements
  - Decision rule requirements include the acceptable probability of making a wrong decision
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
Process

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
**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.
Analytical Procedure Qualification

Experiments

- 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.
Combine uncertainty components

- Combine the different estimates of random variability to get the overall uncertainty estimate
Standard Uncertainty (lower case u)

- Uncertainty (u) is treated as a standard deviation.
- Standard deviations (s) are combined as their variances (v) which is $s^2$.

\[
s_c = \sqrt{(s_1^2 + s_2^2 + \ldots + s_n^2)}
\]

\[
u_c = \sqrt{(u_1^2 + u_2^2 + \ldots + u_n^2)}
\]
Example – Category II Quantitative

<table>
<thead>
<tr>
<th>Analytical Performance Characteristics</th>
<th>Category I</th>
<th>Category II Quantitative</th>
<th>Limit Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>Yes</td>
<td>Yes</td>
<td>*</td>
</tr>
<tr>
<td>Precision</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Specificity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Quantitation Limit</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Linearity</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Range</td>
<td>Yes</td>
<td>Yes</td>
<td>*</td>
</tr>
</tbody>
</table>

*May be required, depending on the nature of the specific test.
Example

- Clinical study requirements
  - 325 to 650 mg
- USP monograph 95.0% to 105.0%
  - Calculates to be 464mg to 512mg
  - Well within clinical study requirements
Decision rule

- Decision Makers decide
  - Acceptable probability of accepting a wrong value is 5%.

- Decision rule
  - The lot of drug product will be considered compliant if the probability of releasing a lot incorrectly is less than 5%.
Assumptions

- For simplicity, we will assume no manufacturing variability
  - There are different procedures to use when the manufacturing variability is significant, but that will not be discussed here

- Determine the TMU
  - Use spreadsheet
  - Assume no bias
<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>2.55</td>
</tr>
<tr>
<td>Lower Limit</td>
<td>95.0</td>
</tr>
<tr>
<td>Upper Limit</td>
<td>105.0</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>% above upper limit</th>
<th>Total outside limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50%</td>
<td>2.50%</td>
<td>5.0%</td>
</tr>
</tbody>
</table>

### Concentration

![Graph showing concentration range with lower and upper limits]
HPLC (0.6 to 1.0%) RSD intermediate precision

<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>1.00</td>
</tr>
<tr>
<td>Lower Limit</td>
<td>95.0</td>
</tr>
<tr>
<td>Upper Limit</td>
<td>105.0</td>
</tr>
</tbody>
</table>

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

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

![Concentration Graph](image)
Performance Characteristics

Acceptance Criteria

<table>
<thead>
<tr>
<th>Range</th>
<th>85 to 115</th>
</tr>
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<tbody>
<tr>
<td>Accuracy</td>
<td>Bias</td>
</tr>
<tr>
<td>Specificity</td>
<td>See Accuracy</td>
</tr>
<tr>
<td>Precision</td>
<td>Repeatability</td>
</tr>
<tr>
<td></td>
<td>Int Precision</td>
</tr>
<tr>
<td>Linearity</td>
<td>Calibration</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
</tr>
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</table>

Combination of Bias and Uncertainty meets target measurement uncertainty and meets the decision rule requirements.
<table>
<thead>
<tr>
<th>Replicate</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
<th>Experiment 5</th>
<th>Experiment 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98.81</td>
<td>100.68</td>
<td>100.51</td>
<td>100.53</td>
<td>98.74</td>
<td>99.15</td>
</tr>
<tr>
<td>2</td>
<td>99.21</td>
<td>100.72</td>
<td>100.44</td>
<td>99.81</td>
<td>99.51</td>
<td>100.86</td>
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<tr>
<td>3</td>
<td>100.41</td>
<td>99.17</td>
<td>99.58</td>
<td>100.47</td>
<td>100.38</td>
<td>100.04</td>
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<tr>
<td>4</td>
<td>99.16</td>
<td>99.85</td>
<td>99.12</td>
<td>99.44</td>
<td>100.98</td>
<td>101.27</td>
</tr>
<tr>
<td>5</td>
<td>100.90</td>
<td>99.46</td>
<td>100.84</td>
<td>99.49</td>
<td>100.43</td>
<td>99.28</td>
</tr>
<tr>
<td>6</td>
<td>99.96</td>
<td>100.85</td>
<td>99.68</td>
<td>99.95</td>
<td>100.42</td>
<td>99.16</td>
</tr>
<tr>
<td>7</td>
<td>98.93</td>
<td>99.34</td>
<td>100.65</td>
<td>99.94</td>
<td>98.85</td>
<td>100.18</td>
</tr>
<tr>
<td>Standard Deviation (s)</td>
<td>0.81</td>
<td>0.72</td>
<td>0.65</td>
<td>0.43</td>
<td>0.87</td>
<td>0.85</td>
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<tr>
<td>Minimum s</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum s</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Add 20 to 30% to TMU

The MU can be multiplied by 20 to 30% to compensate for the variability of estimating the MU.
For our example, using 20%, the TMU becomes

\[
\frac{2.55}{1.2} = 2.1\%
\]
## Bias & Repeatability

<table>
<thead>
<tr>
<th>Target Concentration</th>
<th>85.0</th>
<th>100.0</th>
<th>115.0</th>
<th>85.0</th>
<th>100.0</th>
<th>115.0</th>
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<tbody>
<tr>
<td></td>
<td>84.72246</td>
<td>100.5663</td>
<td>115.052</td>
<td>99.67348</td>
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<td></td>
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<td>100.8293</td>
<td>99.28669</td>
<td>99.56766</td>
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<td>114.5189</td>
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<td></td>
<td>85.26919</td>
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<td></td>
<td>85.11559</td>
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<td>115.7377</td>
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<td>100.1788</td>
<td>100.6414</td>
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<tr>
<td></td>
<td>84.98115</td>
<td>99.94784</td>
<td>115.2321</td>
<td>99.97783</td>
<td>99.94784</td>
<td>100.2018</td>
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<tr>
<td>STDEV</td>
<td>0.611</td>
<td>0.478</td>
<td>0.435</td>
<td>0.719</td>
<td>0.478</td>
<td>0.378</td>
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<tr>
<td>AVG</td>
<td>85.19</td>
<td>99.84</td>
<td>114.98</td>
<td>100.23</td>
<td>99.84</td>
<td>99.98</td>
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<tr>
<td>COUNT</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
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</tr>
<tr>
<td>Degrees of Freedom (DF)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Bias</td>
<td>0.19</td>
<td>-0.16</td>
<td>-0.02</td>
<td>0.23</td>
<td>-0.16</td>
<td>-0.02</td>
</tr>
<tr>
<td>Overall Recovery</td>
<td>100.02</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Overall Bias</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
**ANOVA** ($s_r$, uncertainty component)

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>Column 1</td>
<td>7</td>
<td>701.5754</td>
<td>100.2251</td>
<td>0.516283</td>
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<tr>
<td>Column 2</td>
<td>7</td>
<td>698.8946</td>
<td>99.84208</td>
<td>0.228329</td>
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<tr>
<td>Column 3</td>
<td>7</td>
<td>699.849</td>
<td>99.97842</td>
<td>0.143128</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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</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.52756</td>
<td>2</td>
<td>0.26378</td>
<td>0.891411</td>
<td>0.427423</td>
<td>3.554557</td>
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<tr>
<td>Within Groups</td>
<td>5.326436</td>
<td>18</td>
<td>0.295913</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.853997</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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>

The MS’s do not differ significantly. Both estimate repeatability.
Example of Factors Selected for HPLC Robustness Study

Factors
- extraction heating time,
- extraction temperature,
- HCl concentration,
- extraction volume,
- column temperature,
- flow rate, and
- test sample weight
Critical Variable Identified

Operational control implemented for extraction heating time – require 30 minutes heating time, enter that in SOP, require actual heating time to be recorded

The overall standard deviation is less than TMU of 2.1% so no further experiments are needed

<table>
<thead>
<tr>
<th>Robustness Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
</tr>
<tr>
<td>A extraction temperature</td>
</tr>
<tr>
<td>B flow rate</td>
</tr>
<tr>
<td>C HCl concentration</td>
</tr>
<tr>
<td>D extraction volume</td>
</tr>
<tr>
<td>E column temperature</td>
</tr>
<tr>
<td>F extraction heating time</td>
</tr>
<tr>
<td>G test sample weight</td>
</tr>
</tbody>
</table>

Overall STDEV: 1.81947

n: 8

DF 7
CHAPTER 7
Regression

7.1 Linear Regression

7.1.1 Introduction to Linear Regression

Linear regression is used to establish or confirm a relationship between two variables. In classical statistical theory, linear regression is commonly used in the construction of calibration curves for analytical methods, such as in atomic absorption spectroscopy, where a linear relationship is expected between the analytical response and the concentration of the analyte. Any two data sets of equal size can be plotted on a graph to show the relationship between corresponding pairs of variables. In technical chemistry, the data sets are often extensive and require statistical representation. If there is reason to believe that the scatter is simply due to error and is dependent on the value of the other, the former is known as the dependent variable. The dependent variable is usually plotted on the y-axis of the scatter plot; for the purpose of the calibration curve, the concentration of the analyte is the dependent variable and its value will depend on the concentration of the standard. The linear correlation coefficient measures the strength of the relationship between these two variables and is denoted by r. The object of regression is to establish the relationship between the variables of interest and often to study other aspects of the relationship. The general equation which describes a straight line can be written as:

\[ \sum_{i=1}^{n} (x_i - \bar{x})^2 = \sum_{i=1}^{n} y_i - n \bar{y} \]

where \( \bar{x} \) is the product of the line and \( \bar{y} \) is its y-intercept with the axis. The method of least squares is the linear regression is to estimate the values of a and b. The best fit line is obtained by least squares through linear regression. The line which minimizes the sum of the squared differences between the predicted value of \( \bar{y} \) and the observed value \( y_i \) is known as a residual. The square root of sum of residuals is a known as the residual. This process is known as square error and the only error occurs in the measurement of \( y_i \).

7.1.2 Assumptions in Linear Regression

A straight line best represents linear regression of \( x \) vs. \( y \) to the data on a number of assumption. Subtraction of any of the assumptions will usually require special treatment. The key assumptions are:

- Normality: The errors are normally distributed.
- Linearity: The relationship between \( x \) and \( y \) is linear.
- Homoscedasticity: The variance of the errors is constant.
- Independence: The errors are independent of each other.

Practical Statistics for the Analytical Scientist
A Bench Guide

RSC Publishing
With the uncertainty from linearity

- Is the calibration linear enough?
- How much uncertainty comes from the linearity – from the calibration?
- The uncertainty in the predicted value of $x$:

$$S_{\hat{x}} = \frac{s_{y/x}}{b} \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(\bar{y}_o - \bar{y})^2}{b^2 \sum_{i=1}^{n} (x_i - \bar{x})^2}}$$
### Error in the prediction

*Predictions*

<table>
<thead>
<tr>
<th>$y_o$, response for which $x$, concentration, prediction is desired</th>
<th>Predicted $x$, concentration</th>
<th>Standard error of prediction for this predicted value of $x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>75.02</td>
<td>0.10</td>
</tr>
<tr>
<td>80</td>
<td>80.01</td>
<td>0.10</td>
</tr>
<tr>
<td>90</td>
<td>89.98</td>
<td>0.09</td>
</tr>
<tr>
<td>100</td>
<td>99.95</td>
<td>0.09</td>
</tr>
<tr>
<td>105</td>
<td>104.94</td>
<td>0.09</td>
</tr>
<tr>
<td>110</td>
<td>109.92</td>
<td>0.10</td>
</tr>
<tr>
<td>115</td>
<td>114.91</td>
<td>0.10</td>
</tr>
<tr>
<td>120</td>
<td>119.89</td>
<td>0.10</td>
</tr>
</tbody>
</table>

If the error in the predicted value of $x$ is acceptable or insignificant, the calibration is linear enough.

The standard error is the uncertainty arising from the calibration.

See spreadsheet and references for explanation of formula.
LET’S PUT IT ALL TOGETHER
Acceptance Criteria for Qualification

- Combination of Bias and Uncertainty meets target measurement uncertainty and meets the decision rule requirements
  - Decision rule requirements include the acceptable probability of making a wrong decision

- Decision rule
  - The lot of drug product will be considered compliant if the probability of releasing a lot incorrectly is less than 5%.
Performance Characteristics from 1225

Acceptance Criteria

<table>
<thead>
<tr>
<th>Range</th>
<th>85</th>
<th>to 115</th>
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<tbody>
<tr>
<td>Accuracy</td>
<td>Bias</td>
<td></td>
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<tr>
<td>Specificity</td>
<td>See Accuracy</td>
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<tr>
<td>Precision</td>
<td>Repeatability</td>
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<tr>
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<td>Int Precision</td>
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<td>Calibration</td>
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<tr>
<td>Robustness</td>
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Target Uncertainty

Combination of Bias and Uncertainty meets target measurement uncertainty and meets the decision rule requirements.

Experimental Results

| Bias from Recovery | 100.02 |
| Bias               | 0.02   |
| Uncertainty Components & DF | u | DF |
| Bias Uncertainty   | 0.23  | 44.1 |
| Linearity at 100%  | 0.090 |
| Repeatability      | 0.54  | 18   |
| Int Prec           |       |
| Robustness         | 1.82  | 7    |

Combined Standard Uncertainty | 1.83 | 7.2 |

Rationale: The combined uncertainty uses the robustness and bias uncertainty components. The repeatability is included in the robustness component.

Instruction: Evaluate uncertainty components and combine accordingly. Describe rationale for calculation.
Evaluate the uncertainties

### Experimental Results

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| Combined Standard Uncertainty | 1.83 | 7.2 |

**Rationale:** The combined uncertainty uses the robustness and bias uncertainty components. The repeatability is included in the robustness component.

Bias is insignificant.

Bias uncertainty comes mainly from the uncertainty of the certified reference value. (Not discussed in this presentation.)

Linearity and repeatability are included in each robustness experiment, so they are not used in the combined uncertainty.

Instruction: Evaluate uncertainty components and combine accordingly. Describe rationale for calculation.
Double Count

- Each robustness experiment includes uncertainty from calibration and repeatability.
- Hence, these values were not combined with the robustness uncertainty.

$$\sqrt{+0.23^2 + 0.09^2 + 0.54^2 + 1.82^2} = 1.91$$

- 1.91 is not very different from 1.83 for uncertainty
Useful information

- Knowing the uncertainty for linearity, repeatability, bias, etc. is useful
- The analytical procedure is better understood
- Adequate operational controls can be implemented
Will this analytical procedure produce reportable results that are fit for use?

- **TMU** = 2.1%
- **MU** = 1.8%
- Expect < 0.5% probability of making a wrong decision due to analytical variability
Example from REAL data
Conclusion

- Decision rules and TMU are great tools for making the link to fitness for purpose.
- The acceptance criteria for an analytical procedure qualification can be formulated and assessed using the TMU and decision rule.
Disclaimer

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