Use of Measurement Uncertainty in Testing a Drug Substance

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Eurachem/CITAC Workshop June 2011

Thank You to United States Pharmacopeia for Their Sponsorship and Assistance

The USP Vision Includes:

- A United States Pharmacopeia (USP) Reference Standard and its associated Monograph for every medicine, food ingredient, and dietary supplement in global commerce.
- International recognition, harmonization, and official acceptance of all USP Reference Standards.
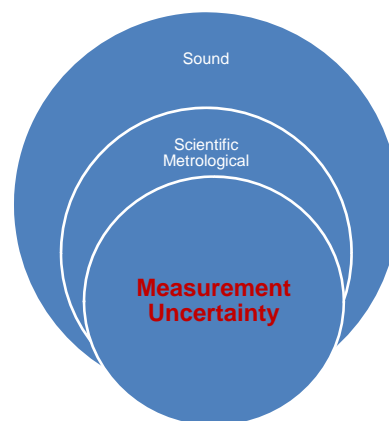

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Metrological Principles

- The Vision includes cutting-edge USP Reference Standards, including Certified Reference Materials, based on sound, scientific, metrological principles.

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USP Initiatives to Meet the Vision

- Documents of the Certified Reference Materials will include uncertainty statements for the assigned values.

- ...a performance-based monograph (PBM) would define only the criteria needed to show that the procedure used is acceptable.
- Measurement Uncertainty is one of the criteria.

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Official Recognition of USP-NF

- The U.S. Federal Food, Drug, and Cosmetics Act designates the USP–NF (National Formulary) as the official compendia for drugs marketed in the United States.
- A drug product in the U.S. market must conform to the standards in USP–NF to avoid possible charges of adulteration and misbranding.
- For these reasons, companies use USP compendial material and meet USP requirements, which will include Measurement Uncertainty.

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A Real Life Example

- This presentation will demonstrate the use of Measurement Uncertainty (MU) in method validation, routine method use, and reporting data for an assay and impurities test of a pharmaceutical Drug Substance.

- The Drug Substance (DS) in this example has a monograph in the United States Pharmacopeia (USP).

Validation ▸ Use ▸ Reporting
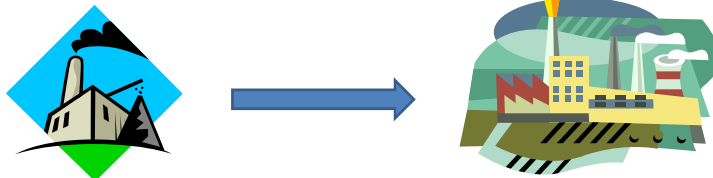
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DS and Impurity A

- This is a general example based on several cases for illustration of the key applications of MU.

  - The Drug Substance itself will be called DS in this example.

  - The impurities will be identified as impurities A, B, C and D.

    - The approach for all impurities is the same, so data will be presented for Impurity A only.

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Product Transfer

- The USP monograph contains a test to determine the concentration of the impurities and to determine whether the DS meets the assay specification of 98.0% to 102.0%.
- The lab had to implement the assay and impurity method to support a product transfer to meet a regulatory filing timeline.

Urgency

- There are many reasons why a method must be implemented quickly.

  - This may be a generic drug and the company needs to be "first to file" to capture the market.
  - The company may be closing a site and needs to transfer the product with its testing to meet a timeline.
  - The method may be needed to meet a compliance commitment.
  - The existing method can not be used, for example, a critical reagent may no longer be available.

Little Time

- The method must be developed, validated, and implemented quickly, sometimes within weeks.

- To do this, the lab can only perform the minimum of development and validation activities.

- MU can help expedite the goal of having an effective method efficiently and effectively and ..... Quickly.

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Specifications

- The test method uses High Performance Liquid Chromatography (HPLC).

- The monograph lists two impurities, Impurity A and Impurity B.

- The specification for assay is 98.0% - 102.0%.

- Impurity A and impurity B have the same specification, Not More than (NMT) 0.15% of the Drub Substance, %DS.

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USP Reference Standards

- USP Reference Standards are available for the Drug Substance and for the two impurities listed in the monograph.
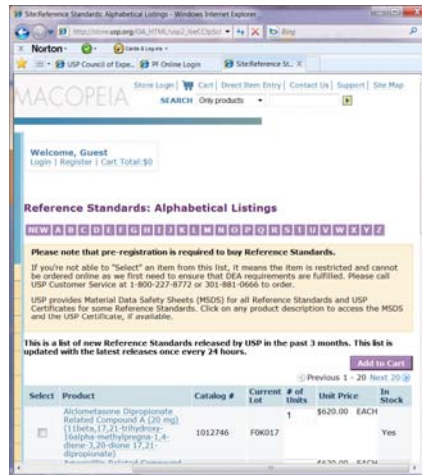
Two Additional Impurities

- Industry is required to ensure they are testing their drugs for all known and possible impurities.
- A literature search revealed that there were two additional impurities, Impurity C and Impurity D, that were not included in the monograph assay and impurities method.
- The lab obtained Reference Standards for these impurities.

A New Method was Needed

- The lab determined that the USP test would not adequately detect Impurities C and D; hence, the lab developed a new method that would detect and quantify all four impurities.
- Since this was a new, in-house method, the lab had to validate the method following the requirements in USP General Chapter <1225> Validation of Compendial Procedures.

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<1225> Validation of Compendial Procedures

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Data Elements Required for Validation

Analytical Performance Characteristics	Category I Assay	Category II Quantitative Impurities
Accuracy	Yes	Yes
Precision	Yes	Yes
Specificity	Yes	Yes
Detection Limit	No	No
Quantitation Limit	No	Yes
Linearity	Yes	Yes
Range	Yes	Yes

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Harmonized with ICH Q2

- The text of the information in General Chapter <1225> harmonizes, to the extent possible, with the Tripartite International Conference on Harmonization (ICH) documents Validation of Analytical Procedures and the Methodology extension text.
- The validation approach is consistent with that of other industries.

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Validation Approach is Defined

- The USP is clear and specific in General Chapter <1225> on how to conduct the validation.
  - It states which analytical performance characteristics need to be included in the validation.
  - The number of determinations is given.
  - Details on how to report the values, such as % Recovery, are specified.

Accuracy <1225>

- Assay accuracy is validated by "application of the analytical procedure to an analyte of known purity (e.g., a Reference Standard)".

- "In the case of quantitative analysis of impurities, accuracy should be assessed on samples (of drug substance or drug product) spiked with known amounts of impurities."

% Recovery & Number of Determinations

- "Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample"

- "The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration)."

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Accuracy as % Recovery (Data Set 1)

	50%	100%	150%
	100.356	99.816	98.703
	100.642	99.891	99.299
	100.822	99.823	99.157
		99.551	
		99.946	
		99.403	
Average	100.61	99.74	99.05
Standard Deviation	0.24	0.21	0.31

- There appears to be a trend in the bias. Since all routine samples will be at the 100% level, we will use that data. When the method is implemented, the accuracy will be closely monitored.

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Accuracy (% Recovery)

- The data for 100% Drug Substance is used to calculate the accuracy.
- The Certificate of Analysis (COA) does not include the uncertainty.
  - Since the COA value is obtained from at least 3 labs often using properly executed procedures in which all sources of bias have been accounted for (i.e., reference measurement procedures), assume the uncertainty of the RM is negligible.

Obtained	Theoretical	Accuracy	u	Degrees of Freedom
99.74	100.0	-0.26	0.09	5

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Precision Studies

- USP <1225> includes the following requirements for testing precision:
  - Repeatability
  - Intermediate precision, also known as ruggedness.
- The data sets for these requirements include several sources of data to estimate uncertainty.
  - For example, the intermediate precision data covers both repeatability and intermediate precision.
- Robustness tests may also include sources of data to estimate uncertainty.

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<1225> Repeatability

- Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment.
- Repeatability should be assessed
  - using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration) or
  - using a minimum of six determinations at 100% of the test concentration.

Repeatability – Data Sources

- Data Set 1 (accuracy) includes three sources.
  - 50%, 100% and 150% concentrations.
- The experiment for intermediate precision, between analysts in this case, includes another source.
  - The data for each analyst is a source of repeatability data.
- The extraction time study is another source.
  - An extraction time study determined that extraction was complete after 16 minutes, so data for 20 and 24 minutes extractions can be used for repeatability.

Repeatability

Data Source	Standard Deviation	Degrees of Freedom
Data Set 1 (50% & 150%)	0.34	4
Intermediate Precision (Between Analysts)	0.18	10
Extraction Study	0.59	4
u (Pooled)	**0.35**	**18**

<1225> Intermediate Precision

- Intermediate precision expresses within-laboratory variation, as on different days, or with different analysts, or equipment within the same laboratory.
- ANOVA is valuable at obtaining the standard deviation for intermediate precision.

USP <1225> Determination of Intermediate Precision

- The precision of an analytical procedure is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation.

- Assays in this context are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result.

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Data for Intermediate Precision

- There are 2 sources of data for intermediate precision uncertainty.
  - ❑ The data from the experiment between analysts.
    - The data is titled "Between Analysts", but also is between HPLCs and columns.
  - ❑ The data from the Robustness study.
    - This includes different mobile phase composition, buffer pH, flow rates on the HPLC, and column temperature.

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Between Analysts

- ANOVA was used to extract the within group standard deviation, repeatability, and between analyst standard deviation.

	Analyst A	Analyst B
1	99.816	98.875
2	99.891	98.770
3	99.823	98.520
4	99.551	98.862
5	99.946	98.774
6	99.403	98.692
Standard Deviation	0.21	0.13
Degrees of Freedom	5	5

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ANOVA Results for Between Analysts Variance Component

- The between analysts component of variance is statistically significant.

- Between Analysts Variance is calculated by

  (MS Between Analyst – MS Within Analyst)
  ――――――――――――――――――――
  # replicate Measurements

  - MS is the Mean Square

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USP <1225> Robustness

- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters.
- Typical variations are the pH of the mobile phase, the mobile phase composition, different lots or suppliers of columns, the temperature, and the flow rate.

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ANOVA Results from Robustness

- The Difference between conditions in the robustness experiment is not statistically significant and is negligible.
- Therefore, all the variability in the robustness study can be attributed to within conditions variability. This is the same as intermediate precision.

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Intermediate Precision/Robustness

- The data for Intermediate precision and robustness are pooled.

	Standard Deviation	Degrees of Freedom
Between Analysts	0.70	1
Robustness (Within Group)	0.88	10
u	**0.82**	**11**

Combined Uncertainty

- The uncertainties are combined to estimate the Total Uncertainty.
  - The Degrees of Freedom were calculated using the Welch-Satterthwaite equation.

Source of Uncertainty	u	Degrees of Freedom
Data Set 1 (Accuracy)	0.09	5
Repeatability	0.35	18
Intermediate Precision	0.82	15
Total u	**0.89**	**11**

Validation Design is Critical

- The uncertainty can be estimated using the minimal experimental design guidelines given in USP <1225>.
- With some changes in the experimental design, even more data would be available to estimate MU.
- The experimental design must be clear.
  - For example, the experiment labelled "Between Analysts" included HPLC's and days.

Replicates Must Be Independent

- Replicates are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result.
- Replicates do not always meet this requirement, or it may be difficult to confirm this independence.
  - For example, the same standard is used for robustness runs. This is not obvious in the report and one has to review the lab notebook for this information.

Using u – Decision Rule

- In practice, a simple decision rule is used in which the Drug Substance passes if a value is obtained within the specification limits of 98.0 to 102.0%.
- Now that the lab has estimated u, it can asses the risk of using this method.
- For this example, with a bias of -0.26% and u= 0.89, 3.2% of future values would fall outside specification, below 98.0%.

Risk Mitigation

- The uncertainty from the robustness study could be considered worst case in that during routine use, these conditions will be tightly controlled. Hence, the uncertainty should be less than obtained in this validation.
- The variation between analysts, HPLCs and days was significant. The lab could ensure analysts are trained and proficient, and ensure the HPLC's are working properly to minimize this variability.

Impurity A – Uncertainty Estimate

- The same experiments for assay were performed for Impurity A, except for an extraction study.
- For the impurity, the uncertainty for the accuracy estimate is relatively large.
- The values are expressed as %Recovery, as were the assay values.
- Being an impurity the absolute concentrations are much lower than for the assay.

Impurity A - u Summary

- The u estimates for each performance characteristic were combined to get the u.
 - ❑ The Degrees of Freedom were calculated using the Welch-Satterthwaite equation.

Source of Uncertainty	u	Degree of Freedom
Accuracy	1.24	5
Repeatability	3.49	14
Intermediate Precision	7.97	15
u	**8.79**	**11**

Specification for Impurity A

- The impurity limit is 0.15% of the Drug Substance, %DS.
- The units for uncertainty must be converted from the units used in the validation, % Recovery, to %DS.
- The uncertainty, converted from %Recovery, is 0.013%DS.
   - u %Recovery * 0.15%DS = 8.79 * 0.15%DS

Guard Band

- A Decision Rule with an acceptance zone below the specification could be established, with g being the expanded uncertainty with k=2.

- g = 0.026%DS. The acceptance zone is <0.12%DS.

Acceptance zone   Rejection zone
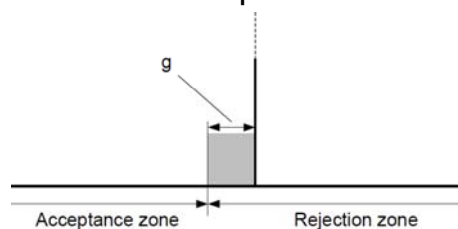
Monitoring Routine Use

- As a result of estimating uncertainty, the lab has enough data to know what aspects of the method to monitor.
  - Control charts could be prepared for repeatability.
  - The performance of different analysts and HPLC's could be closely monitored and controlled.
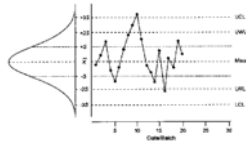
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Number of Replicates

- Labs sometimes validate a method using one test portion (one replicate) and implement the method using the average of two replicates.

- In the absence of knowledge of the Measurement Uncertainty this cautious, conservative approach is taken.

- Knowing the MU, the lab can make an informed decision.

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Implement with One Replicate

- For this method, the largest source of variability comes from the "Intermediate Precision" or between analysts.
- Including two replicates on the same day would not address this variability.
- Knowing this, the lab implements the method using one replicate, saving substantial costs and time.

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Conclusions

- The validation design of USP General Chapter <1225> yields a reliable initial estimate of Measurement Uncertainty.
- Estimating the MU identifies the method steps which yield the greatest variability; the lab understands the method better.
  - Effective controls can be established at these steps immediately upon implementation.

Conclusions

- The risk of implementing the method can be assessed with knowledge and certainty.
- Decision rules can be created using data.
- Unnecessary replication can be avoided, saving time and money.

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The Report Includes the MU!

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