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Statistical evaluation of analytical data and analytical method validation

(lecture)

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Requirements for analytical method

- Selectivity ↑
- Sensitivity ↑
- Reliability ↑
- Sample preparation and handling ↓
- Sample throughput ↑, analysis time ↓
- Economic parameters / cost ↓
- Automatization ↑
- Miniaturization

Evaluation of analytical data (statistic analysis)

- Data set
- Mean The mean, $\bar{X}$, is the numerical average for a data set. We calculate the mean by dividing the sum of the individual values by the size of the data set, where X_i is the i-th measurement, and n is the size of the data set.

$$\bar{X} = \frac{\sum_i X_i}{n}$$

- Standard deviation The standard deviation, s, describes the spread of a data set's individual values about its mean, where X_i is one of n individual values in the data set, and $\bar{X}$ is the data set's mean value. Frequently, the relative standard deviation, s_r, is reported. The percent relative standard deviation, $\%s_r$, is $s_r \times 100$.

$$s = \sqrt{\frac{\sum_i (X_i - \bar{X})^2}{n-1}}$$

$$s_r = \frac{s}{\bar{X}}$$

- Error (Affecting Accuracy) Accuracy is a measure of how close a measure of central tendency is to the expected (i.e. true) value, μ. We can express accuracy as either an absolute error, e, or as a percent relative error, $\%e_r$.

$$e = \bar{X} - \mu$$

$$\%e_r = \frac{\bar{X} - \mu}{\mu} \times 100$$

http://www.asdlib.org/onlineArticles/ecourseware/Analytical%20Chemistry%202.0/Text_Files.html

WHAT IS VALIDATION?

- Validation is defined as the process of demonstrating that the analytical procedure is suitable for its intended purpose
  - Identification
  - Determination of impurities
  - Assay

WHY VALIDATION?

- The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal.

- Validation of analytical methods is also required by most regulations.

Validation

FDA-guidelines:

Validation is establishing **documented evidence which provides a high degree of assurance that a specific process will consistently** produce a product meeting its **pre-determined specifications and quality attributes**

EU-guidelines

Action of **proving, in accordance with GMP-principles that any** procedure, process, equipment, material, activity or system **actually** leads to the **expected results**

What Methods to be Validated?

Defined for:

- identification
- quantitative tests for content of impurities
- limit tests for control of impurities
- quantitative tests for active moiety in drug substances and drug products

Referred to:

- dissolution testing
- particle size determination (drug substance)

PARAMETERS

1. Linearity and Range
2. Specificity
3. Precision
4. Accuracy
5. Limit of Detection
6. Limit of Quantitation
7. Robustness

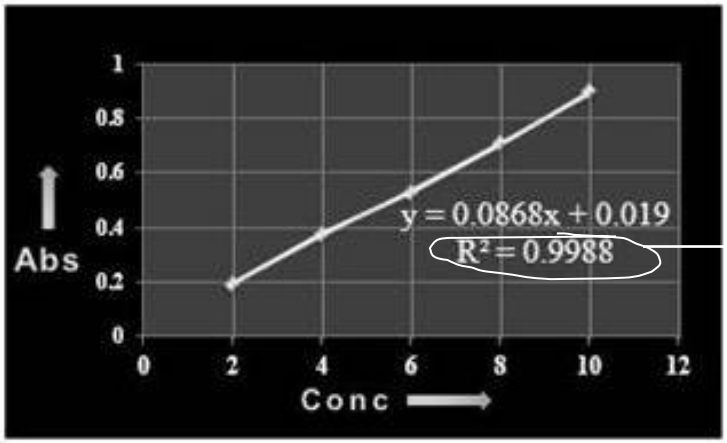
Recommended Validation characteristics of various Types of Tests

Type of tests/Characteristics	Identification	Testing for impurities		Assay/ Dissolution	Specific Tests
		Quantitative	Limits		
Accuracy	-	+	-	+	+
Precision-repeatability	-	+	-	+	+
Precision-Intermediate precision (Ruggedness)	-	+	-	+	+
Specificity	+	-	+	+	+
Detection limit	-	+	+	-	-
Quantitation Limit	-	+	-	-	-
Linearity	-	+	-	+	-
Range	-	+	-	+	-
Robustness	-	+	-	+	+

1. LINEARITY AND RANGE

- Ability to obtain test results that are directly (or by a well-defined mathematical transformation) proportional to the concentration of analyte in samples within a given range.

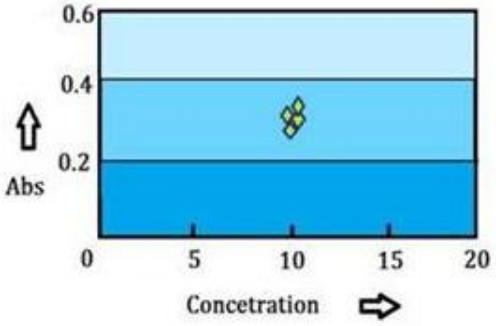
- The range of an analytical procedure is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with a suitable level of precision, accuracy, and linearity

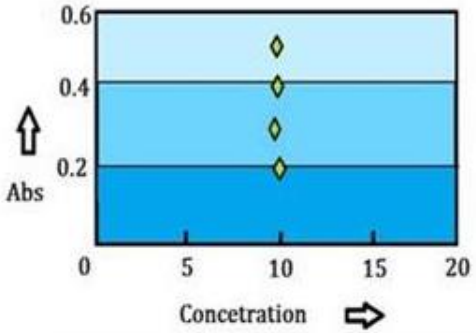


Determination coefficient

2. PRECISION

- The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.





Concentration	Absorbance
10 µg/ml	0.28
	0.31
	0.29
	0.30
Mean	0.29

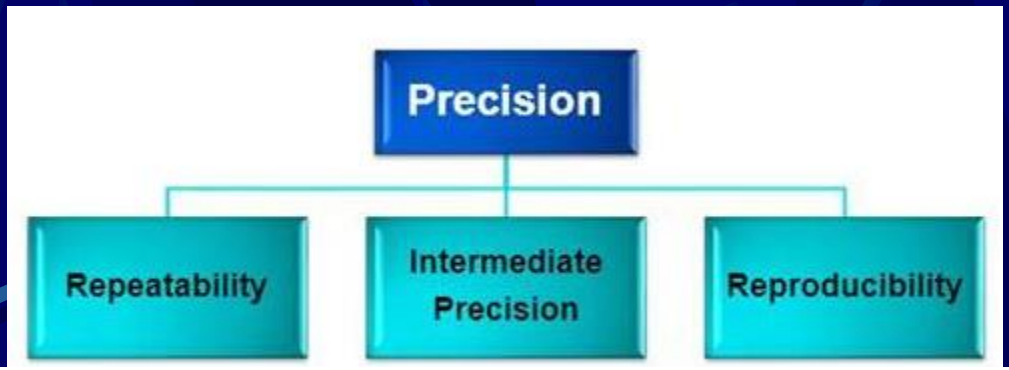
Less Variation
High Precision

Concentration	Absorbance
10 µg/ml	0.21
	0.32
	0.39
	0.53
Mean	0.36

More Variation
Low Precision

variation

precision



1. Repeatability

- Repeatability expresses the precision under the same operating conditions over a short interval of time.
- Repeatability should be assessed using a minimum of 9 determinations covering the specified range.

2. Intermediate Precision

- Intermediate precision expresses variations within laboratories, such as different days, different analysts, different equipment, and so forth

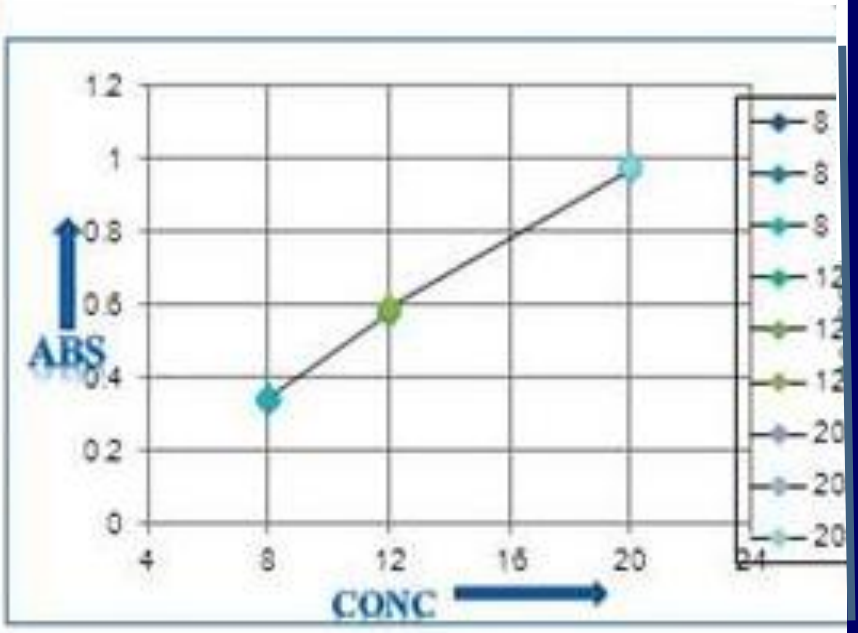
3. Reproducibility

- Reproducibility expresses the precision between laboratories. It is assessed by means of an inter-laboratory trial. (Defined as ruggedness in USP, ISO 17025)

Precision

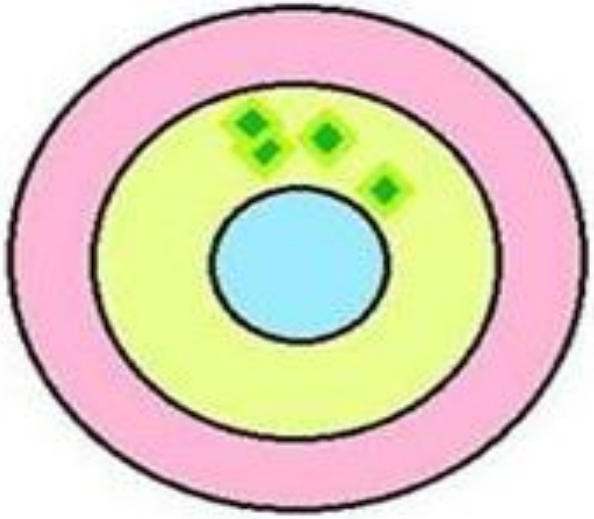
- Following parameters should be reported:
  a. Standard deviation
  b. Relative standard deviation (coefficient of variation)

Concentration µg/ml	Absorbance	SD & % RSD
8	0.337	0.00041, 1.223%
	0.348	
	0.341	
12	0.575	0.0106, 1.815%
	0.583	
	0.596	
20	0.967	0.0091, 0.933%
	0.985	
	0.978	



3. Accuracy

- Closeness of agreement between the conventional true value / an accepted reference value and the value found.



High Accuracy
(Less Precision)



Less Accuracy
(High Precision)

Accuracy (in quantitation experiments)

- Assessed on samples (drug substance/drug product) spiked with known amounts of impurities/drugs.

- ✓ Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure).

- ✓ Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value.

4. Limit of Detection & Limit of Quantitation

- Limit of Detection:
  - It is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated.

- Limit of Quantitation:
  - It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Determination of LOD & LOQ

Limit of Detection

- **Method**
  - ✓ Based on visual evaluation
  - ✓ Based on standard deviation of response and slope

LOD = 3.3 σ / Slope

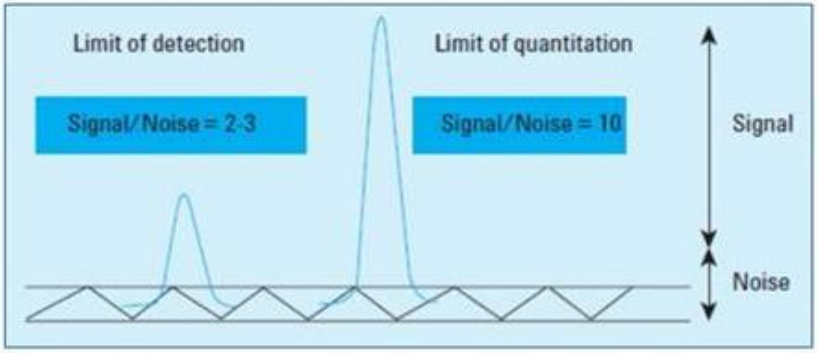
  - ✓ Signal to noise ratio 2:1 or 3:1

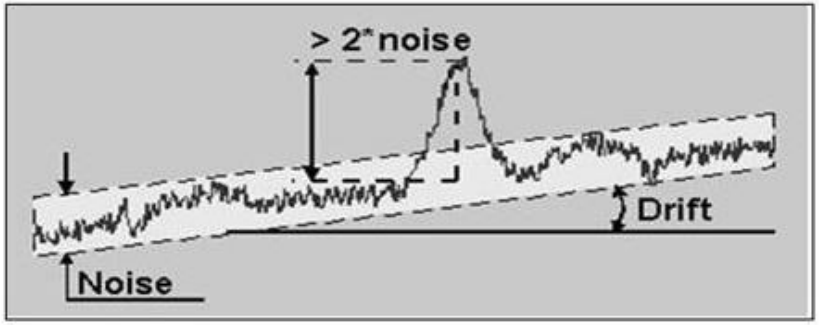
Limit of Quantitation

- **Method**
  - ✓ Based on visual evaluation
  - ✓ Based on standard deviation of response and slope

LOD = 10 σ / Slope

  - ✓ Signal to noise ratio 10:1





6. SPECIFICITY / SELECTIVITY

- The ability to detect the analyte of interest in the presence of interfering substances (typically impurities, degradants, matrix).

1. Identification

- Suitable identification tests should be able to discriminate between compounds of closely related structures which are likely to be present.
- The discrimination of a procedure may be confirmed by obtaining positive results from samples containing the analyte, coupled with negative results from samples which do not contain the analyte.
- The identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained.

7. Robustness

- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

- If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure, such as:
  - Use solution within 24 hours
  - Maintain temperature below 25 degrees

- In the case of liquid chromatography, examples of typical variations are:
  - ✓ influence of variations of pH in a mobile phase
  - ✓ influence of variations in mobile phase composition
  - ✓ different columns (different lots and/or suppliers)
  - ✓ temperature
  - ✓ flow rate

- In the case of gas-chromatography, examples of typical variations are:
  - ✓ different columns (different lots and/or suppliers)
  - ✓ temperature
  - ✓ flow rate

Example

Optimized value: pH 3.0
Deliberate variation: pH 2.9-3.1
Elution time changes: <5.5%
Analytic. signal changes: <4.0%
etc.

CONCLUSION

- When the method is properly validated consistent, reliable and accurate results are obtained. Also, Validation of analytical methods is also required by regulations. Hence it is very important to validate any analytical method that has been developed.

Used and recommended literature

ICH harmonised tripartite guideline validation of analytical procedures ICH Q2A: Definitions and terminology

ICH harmonised tripartite guideline validation of analytical procedures ICH Q2B: Methodology

Method Validation in Pharmaceutical Analysis, A guide to best practice, Edited by Joachim Ermer, John H. McB Miller, Wiley- VCH

- http://www.authorstream.com/Presentation/aSGuest132325-1390320-amv-2007/
- https://www.slideshare.net/sdan/analytical-method-validation-45722369
- Mikuš, Peter; Maráková, Katarína: HYPHENATED ELECTROPHORETIC TECHNIQUES IN ADVANCED ANALYSIS, KARTPRINT, Bratislava, 2012