Analytical method validation

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Analytical method

- Goal
  - consistent, reliable and accurate data
- Validation analytical method
  - serve the goal of analytical method
  - Its data reveals the quality, reliability and consistency of analytical method
  - A important part in good analytical practice; GLP, ISO 17025, ….

Analytical method validation

- When it need to validated, verified or revalidated
  - Before initial use in routine testing
  - Transferred to another lab
  - Change the condition or some method parameters
  - Change the scope outside from original method
- Require to obtain quality data
  - Quality system (Method, Instrument, Reference, Personnel, environment, statistical evaluation,…)
  - USP <1058> Analytical Instrument Qualification

References

- USP
  - <1225> validation of compendial procedures
  - <1226> verification of compendial procedures
- US-FDA: 2 Guidance for Industry; Chemistry and Bioanalytical validation
- ICH: Q2A (Definition & teminology) and Q2B (method)
- IUPAC: “Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis”.
- EURACHEM: developed for ISO/IEC (good source for biopharmaceutical lab
- AOAC: A technical verification of analytical method for ISO 17025 accreditation
- LGC, In-House Method Validation: A Guide for Chemical Laboratories

Regulation and Quality standard

- US-FDA
  - Current Good Manufacturing Practices (cGMP)
  - GLP regulation
  - FDA's regulation for BA and BE Requirement
- PIC/S and EU
  - PIC/S or EU GMP
- ICH: GMP guide
- USP
- ISO/IEC 17025:2005 (5.4.5)

Parameters

- Accuracy
- Precision/ruggedness
- Specificity/sensitivity
- Detection limit (DL, LOD)
- Quantitation limit (QL, LOQ)
- Linerity
- Range
- Robustness
- System suiability

Accuracy

- USP
  - "The closeness of the result obtained by the method to the true value."
- ICH
  - "The closeness of the result obtained by the method to a value that is accepted as conventionally true value or as a reference value."

Accuracy

- Assay for drug substance or impurity
- Reference standard
- Determination
  - Testing of Reference Standard
  - Spike in synthetic mixtures of drug product
  - Standard addition (spiked sample)
  - *in the range of procedure
  - Calculated as % recovery of spiked amount or difference bet the mean and accepted true value plus confidence intervals

Accuracy

- ICH recommendation
  - A minimum of 9 determinations over a minimum of three concentration levels covering the specified range
  - such as 3 conc. *3 replicates of each concentration

Recovery

Level of Method (%)	Amount added (mg)	Amount Found (mg)	Recovery (%)	Statistical analysis
50	25.16	25.06	99.6	Mean 99.9 RSD 0.3
50	25.13	25.06	100.1	
50	25.12	25.06	100.1	
100	50.03	50.16	100.3	Mean 100.0 RSD 0.3
100	50.17	49.87	99.4	
100	50.37	50.47	100.2	
100	50.35	50.35	100.0	
100	50.43	50.38	99.9	
100	50.75	50.45	100.2	
150	75.13	74.43	99.1	Mean 99.4 RSD 0.3
150	75.24	75.01	99.7	
150	75.12	74.67	99.4	

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Comparison to Reference method

Sample	Test Method	Reference Method	Difference
1	91.5	90.3	1.2
2	90.5	90.5	0.0
3	95.2	94.2	1.0
4	90.8	91.6	-0.8
5	96.1	95.7	0.4
		Avg.	**0.36**
		s.d.	**0.80**

- **no significant difference at $p < 0.05$**

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Precision

- Assay for drug substance or impurity
- USP
  - Degree of agreement among individual test results
  - Repeat assay to multiple sampling of homogeneous sample
- Determination
  - Assay a sufficient number of aliquots of a homogeneous sample
  - SD or RSD

Precision

- Practical consideration
  - Sometime depend on instrument efficiency
  - The acceptance criteria for specification should be considered as maximum acceptance criteria to obtain a reliable result (reduce Out-Of Specifications)

Precision

- Repeatability/ intra-assay precision:
  - same analyst within lab over the short period
- Intermediate precision
  - variations within laboratories, such as different days, different analysts, different equipment,
- Reproducibility:
  - different laboratory using the same standarization methadology
- Ruggedness (Reproducibility and intermediate precision)

Repeatability Precision

- ICH recommendation
  - A minimum of 9 determinations over a minimum of three concentration levels covering the specified range
  - Or a minimum of 6 determinations at 100% of the test concentration

Intermediate Precision

- objective
  - to verify that in the same laboratory the method will provide the same results
- Inconsistencies results
  - different operators
  - inconsistent working practice
  - different instruments
  - standards and reagents from different suppliers
  - columns from different batches
  - a combination

Reproducibility Precision

- objective

  to verify that method will provide the same results in different laboratories

- Important for method that use in different laboratory
- US-FDA: at least 2 laboratories
- AOAC protocal: 8 samples, 8 laboratories

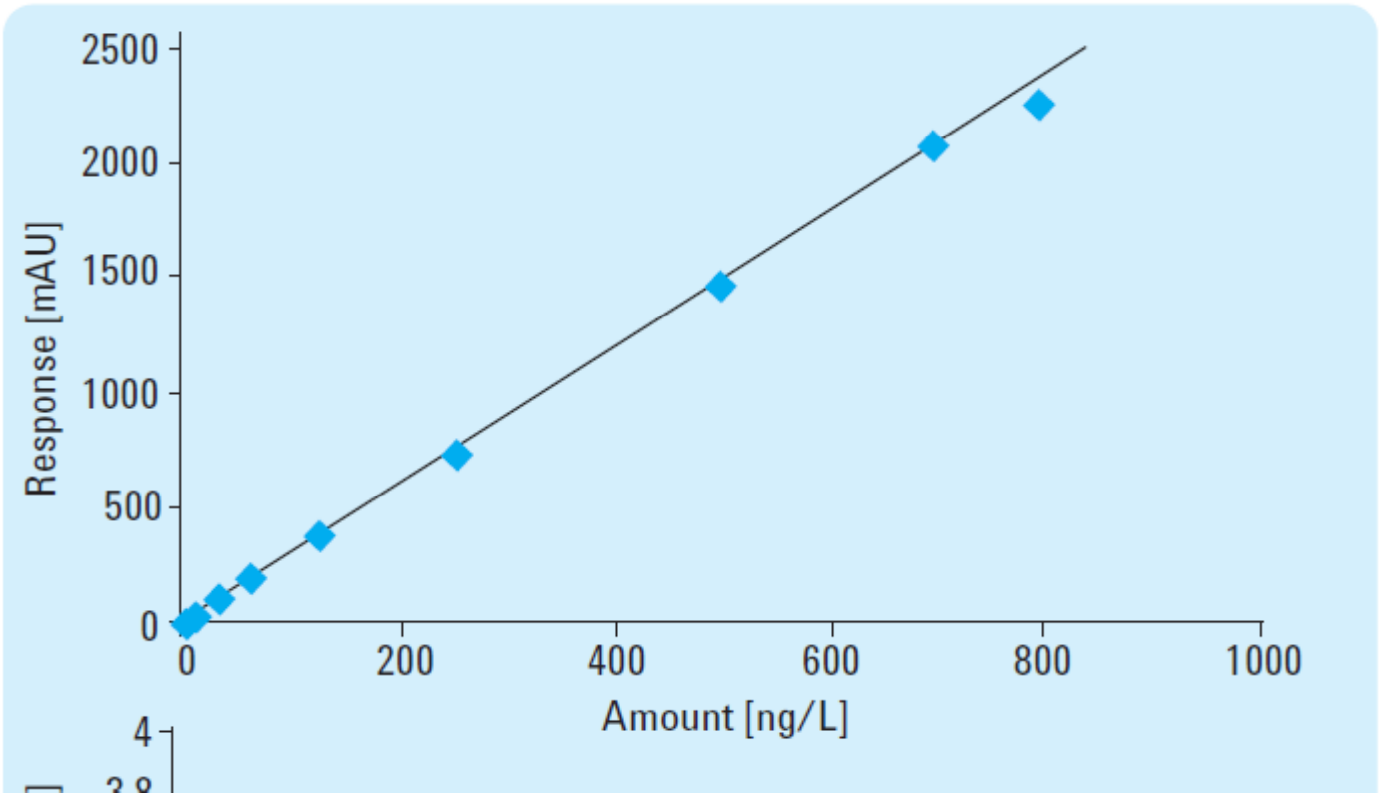
Ruggedness

- Reproducibility with the same samples using different laboratories, analysts, days, reagent lots (same brand), and environmental conditions

- should be evaluated across the specified Quantitation range of the method

linearity

- Refers to the linearity of relationship of concentration and assay measurement
- Directly or by a well-defined mathematical transformation (log, squre root, or reciprocal,…)
- Regression
  - Statistical evaluation of correlation coefficient, y-intercept, slope, and residual sum of squares of regression line
- Non-linear model can be accept
- ICH: at least five concentration



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Range

- The intervals between the upper and Lower levels of analytes
- To be determine in precision, accuracy and linearity
- ICH
  - For assay test, requires the minimum specified range from 80% to 120% of the test concentration
  - For impurity, form LOD (or 50% specification) to 120% specification
  - For Content Uniformity, from 70% to 130% of the test concentration

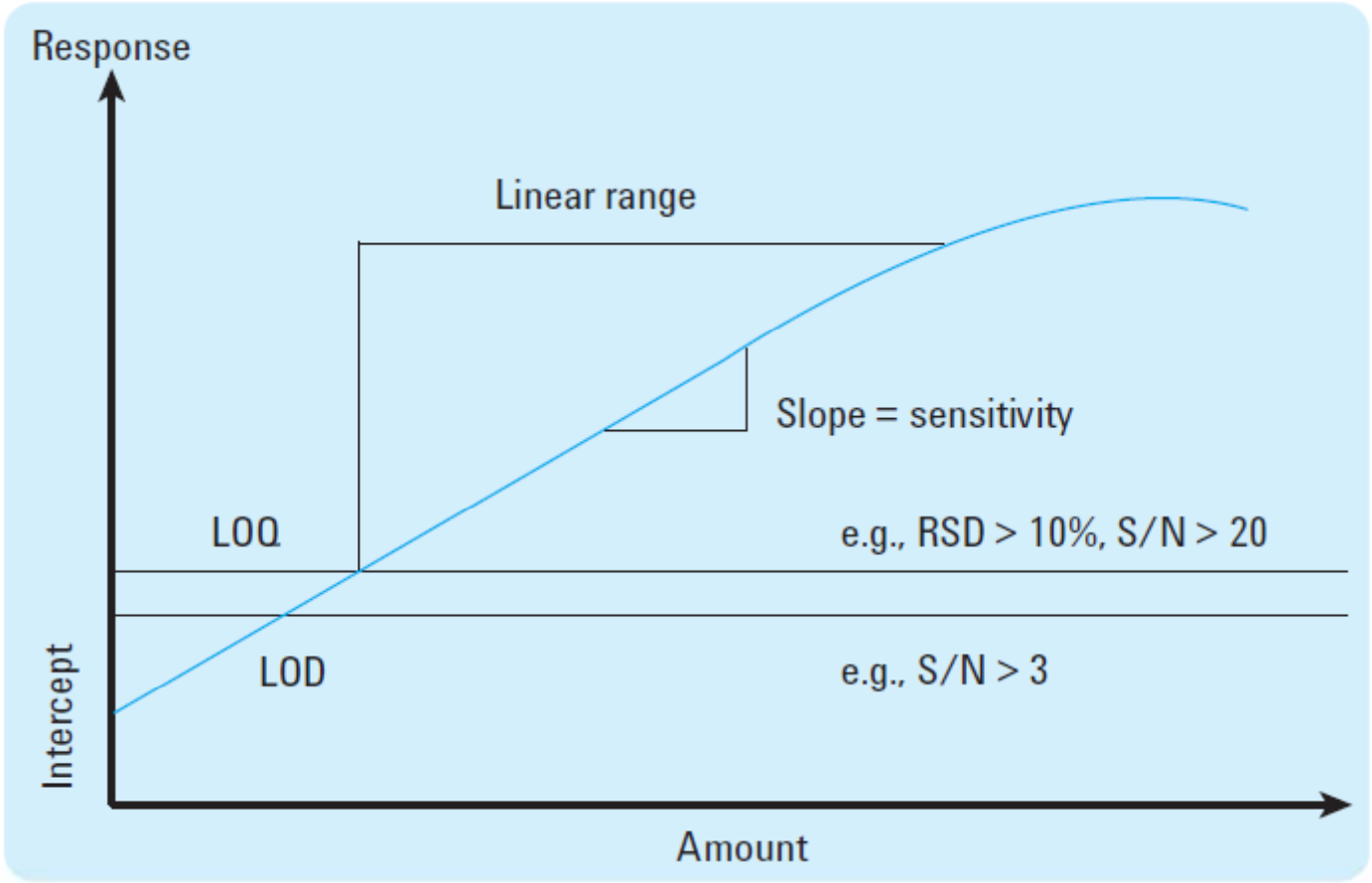

Figure 6
Definition for linearity and range.

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Detection Limit/ Quantitation Limit

- Detection limit (DL, LOD)
  - Characteristic of limit test
  - Determination: noninstrumental procedures
  - Acceptance criteria:
    - signal to noise ratio is 2:1 or 3:1
- Quantitation limit (QL, LOQ)
  - Characteristic of quantitative assay
  - Determination: noninstrumental procedures
  - Acceptance criteria:
    - signal to noise ratio is 10:1

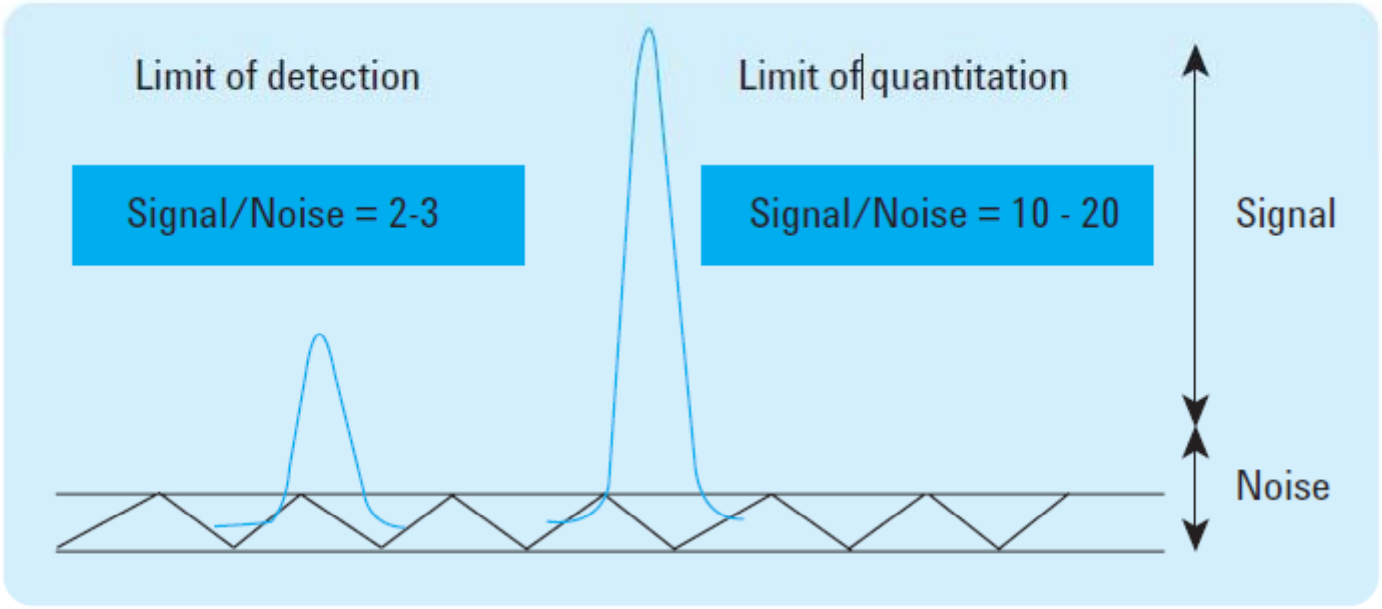

Figure 7
Limit of detection and limit of quantitation via signal-to-noise.

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Specificity/Sensitivity

- **ICH Q2A and USP**
  - the ability to assess unequivocally the analyte in the presence of components that may be expected to be present; impurities, degradation products, and matrix components
- IUPAC, AOAC:“Selectivity” reserving “Specificity”

Specificity/Sensitivity

- **Identification**
  - to ensure the identity of the analyte
- **Purity Test**
  - accurate statement of the content of impurities of an analyte (related substances, heavy metals, residual solvents, etc.)
- **Assay**
  - an exact result, which allows an accurate statement on the content of potency of the analyte in a sample

- From USP

Specificity/Sensitivity

- Some analytical procedures are not sufficiently specific for the intended purpose
  - Assay by titration
  - Identification by UV absorbance
- A combination of two or more analytical procedures is recommended to achieve sufficient specificity

Robustness

- a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters
- Provides an indication of the reliability of the method during normal usage
- method parameters are varied within a realistic range, and the quantitative influence of the variables
- method parameters, such as pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition
- It should be considered early in the development of a method

System Suitability

- Ensures that both methodology and instrumentation are performing within expectation prior to the analysis of the test samples
- Should be monitored during run time to verify that criteria remain realistic and achievable

Data element Required for validation

Analytical task	Identification	Impurity testing Quantitative	Limit tests	Assay
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Intermediate precision	No	Yes	No	Yes
Reproducibility	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
Limit of detection	No	No	Yes	No
Limit of quantitation	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

Figure 10
ICH validation characteristics.

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Data element Required for validation

Analytical Task	Assay Category 1	Assay Category 2		Assay Category 3
		Quantitative	Limit tests	
Accuracy	Yes	Yes	*	*
Precision	Yes	Yes	No	Yes
Specificity	Yes	Yes	Yes	*
Limit of detection	No	No	Yes	*
Limit of quantitation	No	Yes	No	*
Linearity	Yes	Yes	No	*
Range	Yes	Yes	*	*
Ruggedness	Yes	Yes	Yes	*

Category 1: Quantitation of major components
Category 2: Impurities
Category 3: Performance characteristics
* May be required, depending on the nature of the specific test

Figure 11
USP validation characteristics.

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