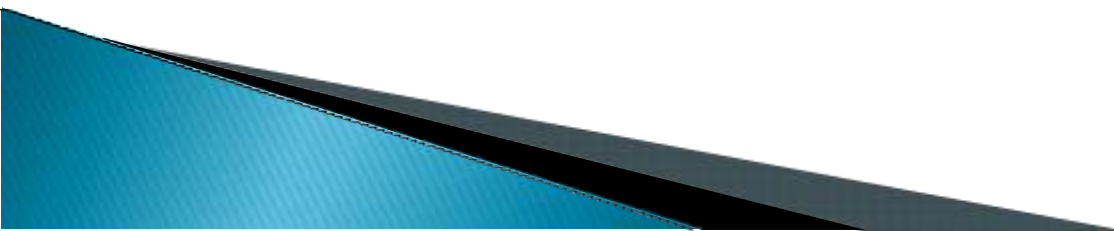


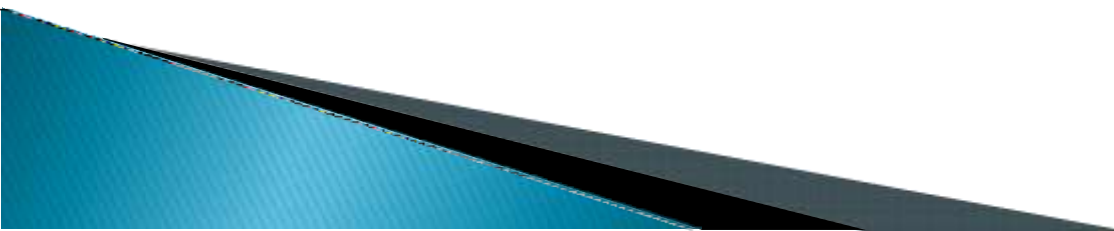
Analytical Method Validation [AMV]

Basic Concepts, Principles and Applications

Why Analytical Method need to be Validated.

- ▶ Analytical methods are used to test and ensure the Quality of Drug substances / Drug products
 - ▶ Analytical methods are followed from Official Pharmacopeia or developed by users.
 - ▶ How can assure the analytical methods are suitable for it purpose.
 - ▶ Hence Analytical Method has to be validated or confirm its suitability?
- 

Method Validation & Requirement

- ❑ Method validation is the process of planned and systematic study to confirm that it is suitable for its intended use.
 - ❑ Method is reliable, accurate & reproducible
 - ❑ Pharmaceutical Industries governed by rules & regulations.
 - ❑ Method validation is mandatory as per regulatory agencies.
- 

Guidance for Method Validation

- CDER : "Analytical Procedures and Method Validation" Draft guidance August 2000
- **ICH:** Validation Of Analytical Procedures: Text And Methodology **Q2(R1) 2005.**
- USP 41: General Chapter <1225> Validation of Compendial Methods



Types of Analytical Procedures

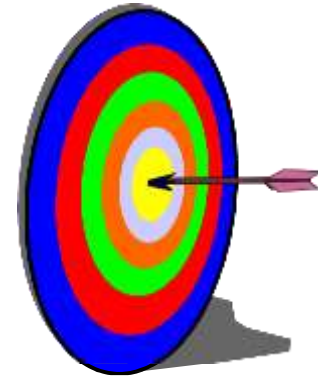
Four most common types of analytical procedures:

- Identification tests - Eg FTIR spectrum
- Quantitative tests for impurities: Eg. 10ppm to 2%
- Limit tests for the control of impurities: Eg < 100ppm
- Quantitative tests of the active substance - Assay / purity : Eg 99-101%

Validation Parameters/characteristics

ICH Q2R1

- Accuracy
- Precision
 - Repeatability
 - Intermediate Precision
- Specificity
- Linearity
- Range
- Detection Limit
- Quantitation Limit



Characteristics to be validated

| Analytical procedure <i>Characteristics</i> | Identification | Tests for impurities | | Assay |
|--|----------------|----------------------|------------|------------|
| | | Quantitative | Limit test | |
| Accuracy | No | Yes | No | Yes |
| Precision- Repeatability | No | Yes | No | Yes |
| Intermediate precision | No | Yes | No | Yes |
| Specificity | Yes | Yes | Yes | Yes |
| Limit of Detection | No | Yes | Yes | No |
| Limit of Quantitation | No | Yes | No | No |
| Linearity | No | Yes | No | Yes |
| Range | No | Yes | No | Yes |
| Robustness * | No | Yes | No | Yes |

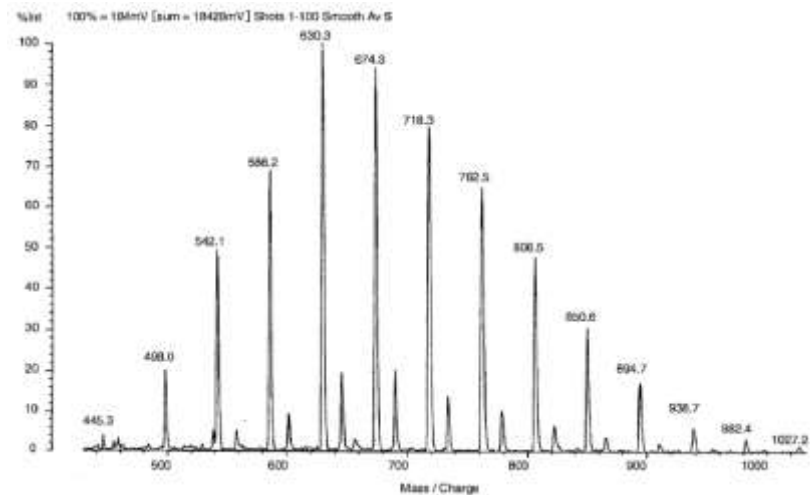
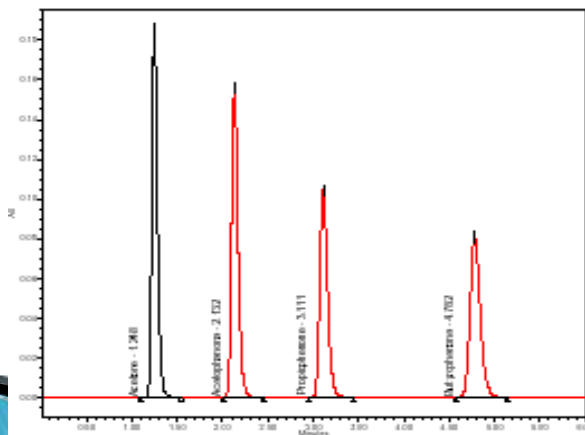
Analytical Method Validation

Specificity

Ability of an analytical method to measure the analyte free from interference from components.

Example:

- HPLC/ GC: well separation of all peaks. Drug from Impurities
- Peak purity- No overlapping of peaks / co elution

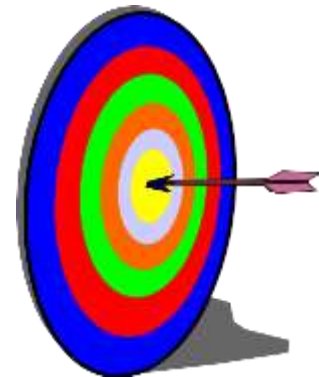


Analytical Method Validation

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

The accuracy is closeness between the experimental value and true value.



Analytical Method Validation

Assay:

Test the assay at 80%, 100% & and 120%

Acceptance criteria:

Recovery : 99.0-101.0%

% RSD should be less than 2.0.



Impurities –Related substances:

Test impurities at 50% – 150% to the limit value.

Acceptance criteria:

Recovery 90.0% to 110.0%

% RSD should be less than 10.0%.

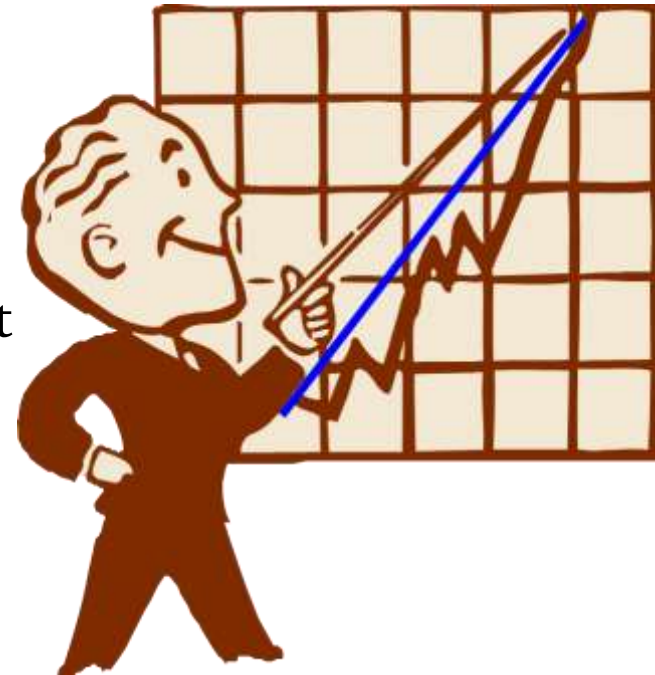
Analytical Method Validation

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity :should be proportional response to analyte concentration.

- ▶ Experiment: Detection level to 150% to limit
Acceptance criteria:
 - Plot Linear Regression ($y = mx + c$)
 - Linear regression $r^2 > 0.98$



Analytical Method Validation

Range:

- ▶ The range of an analytical procedure is the interval **between the upper and lower concentration** (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that **the analytical procedure has a suitable level of precision, accuracy and linearity.**
 - For Drug Substance & Drug product Assay
 - **80** to **120%** of test Concentration
 - For Impurities:
 - LOQ Level to **120%** of Limit

Analytical Method Validation

Limit of Detection

Lowest amount of analyte in a sample that can be detected

- Estimated by Signal to Noise Ratio of 3:1 or
- $3.3 \times \text{Standard Deviation} / \text{Slope}$

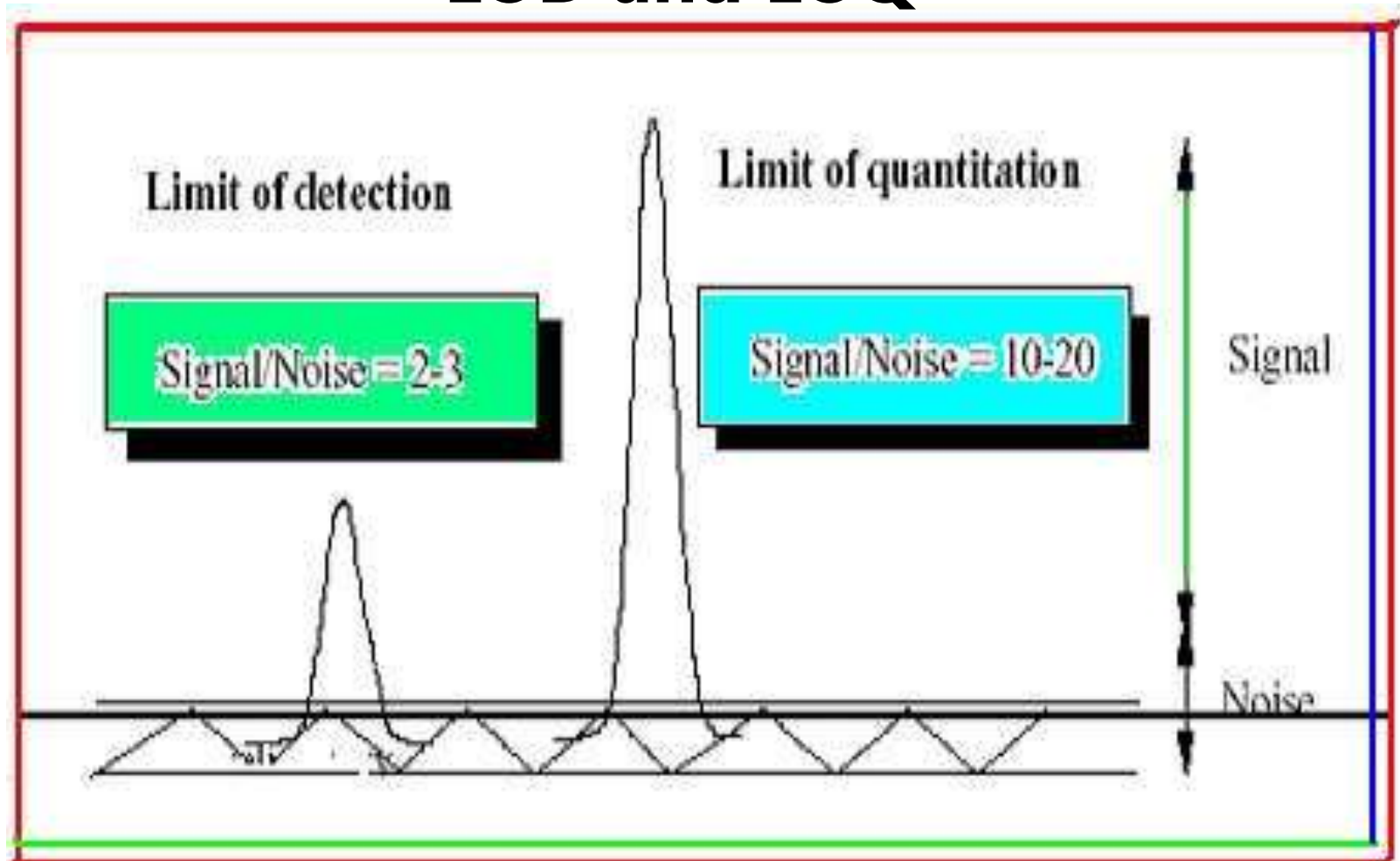
Limit of Quantitation:

Lowest amount of analyte in a sample that can be quantified with suitable accuracy and precision.

- Estimated by Signal to Noise Ratio of 10:1. or
- $10 \times \text{Standard Deviation} / \text{Slope}$

Analytical Method Validation

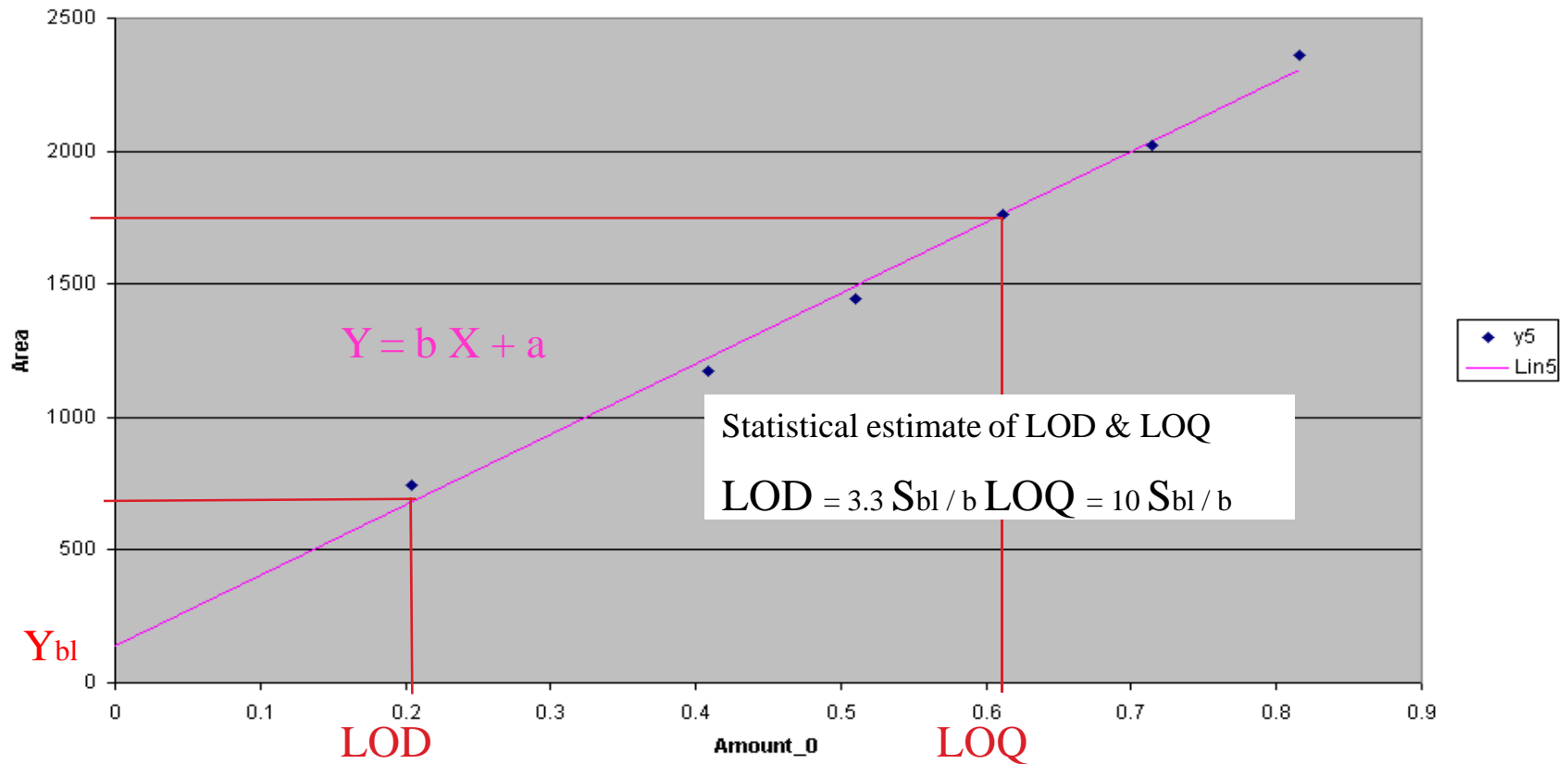
LOD and LOQ



Analytical Method Validation

LOD and LOQ

3.3X Standard Deviation / Slope Limit of Detection



Analytical Method Validation

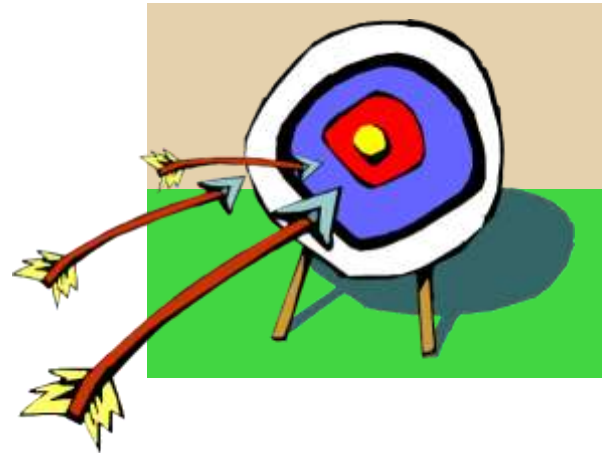
Precision:

Closeness of agreement (degree of scatter) between a series of measurements obtained from multiple testing under the prescribed conditions.

Precision to be established :

Repeatability, Intermediate precision and Reproducibility.

- ▶ The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation



Analytical Method Validation

Repeatability

- ▶ Repeatability expresses the precision under the same operating conditions over a short interval of time.

Intermediate precision

- ▶ Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Reproducibility

- ▶ Reproducibility expresses the precision between laboratories
- 

Analytical Method Validation

| Test | Day 1 | Day 2 | Analyst-1 | Analyst-2 |
|--------------|---------------|--------------|---------------|--------------|
| 1 | 100.3 | 99.8 | 100.4 | 99.6 |
| 2 | 100.5 | 99.9 | 100.2 | 99.9 |
| 3 | 100.1 | 98.9 | 100.3 | 98.9 |
| 4 | 100.3 | 99.4 | 99.8 | 100.1 |
| 5 | 100.5 | 99.7 | 99.9 | 99.7 |
| 6 | 100.7 | 99.6 | 99.6 | 99.8 |
| Mean | 100.40 | 99.55 | 100.03 | 99.67 |
| % RSD | 0.21 | 0.36 | 0.31 | 0.41 |
| | | 0.36 | 0.31 | 0.41 |

Analytical Method Validation

Robustness:

Analytical procedure is capable to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Examples of typical variations in LC

- pH of mobile phase (5.0 ± 0.2)
- Mobile phase composition (± 10 % of solvent)
- Different columns (different lots and/or suppliers)
- Temperature ($30 \pm 2^\circ \text{C}$)
- Flow rate ($1.5 \text{ ml} \pm 0.1 \text{ ml}$)
- ▶ Comparison results under differing conditions with under normal

- ▶ www.fda.gov
- ▶ www.fda.gov/cder/
- ▶ www.usp.org
- ▶ www.ich.org
- ▶ www.pharmweb.net

Questions?

