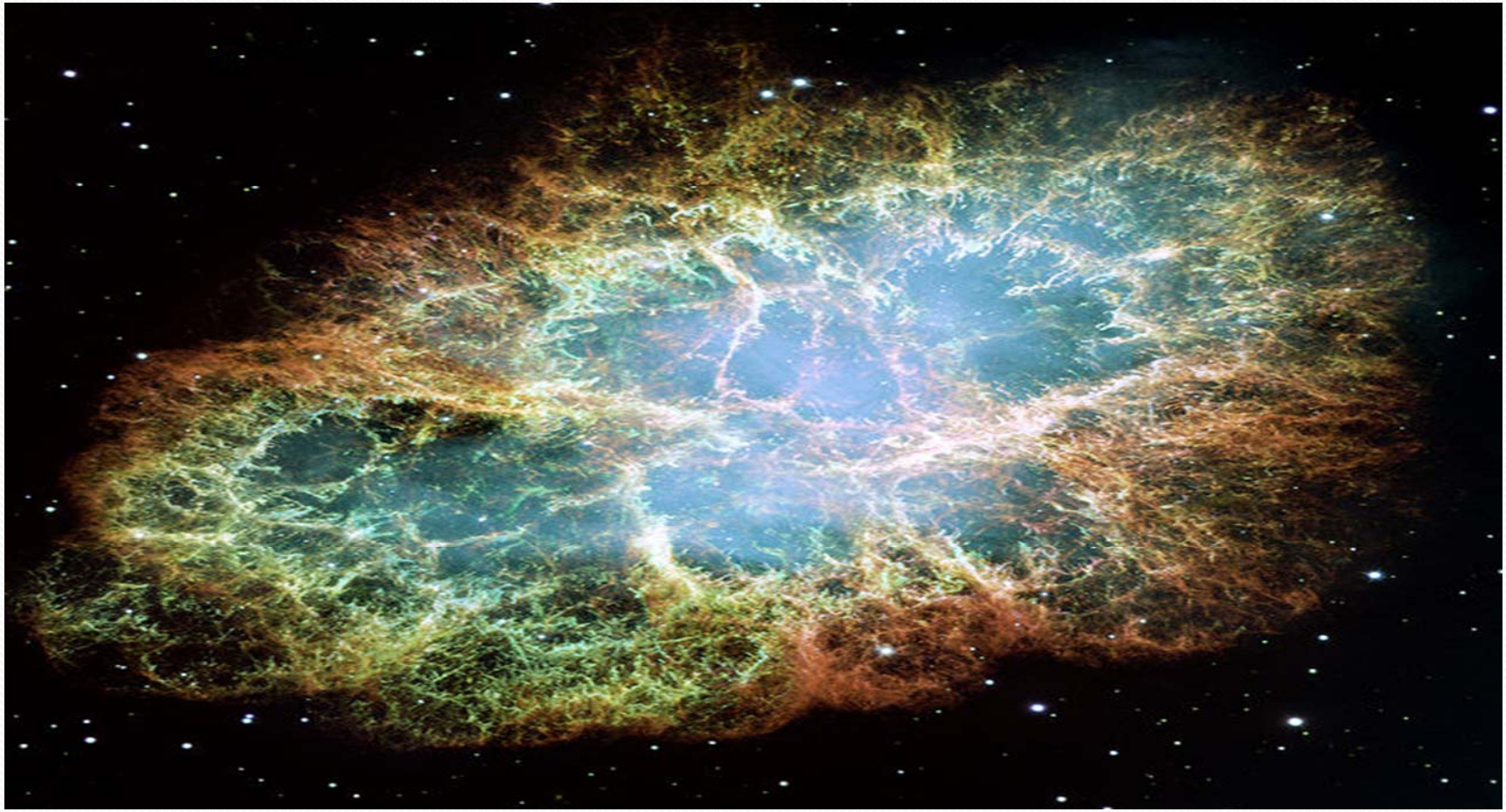
اللَّهُ نُورُ السَّماواتِ وَ الْأَرْض



Validation of Analytical Methods

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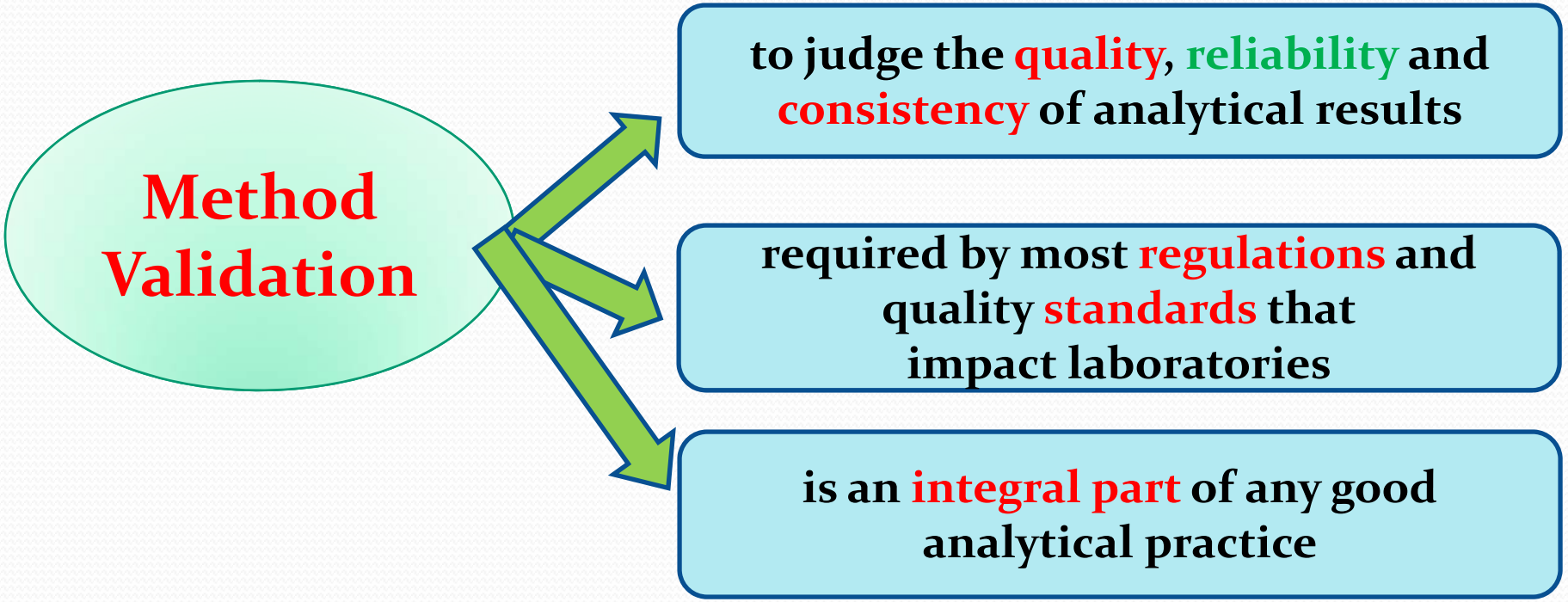
Why Method Validation?

- **Objective of any analytical measurement is:**

  to obtain consistent, reliable and accurate data



When Method Validation?

Analytical methods need to be validated, verified, or revalidated in the following instances:

- Before initial use in routine testing
- When transferred to another laboratory
- Whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or samples with a different matrix) and the change is outside the original scope of the method.

International regulatory bodies and their guidelines

Body	Full name	Guidance on
Eurachem	Focus for Analytical Chemistry in Europe	Method validation
CITAC	Cooperation of International Traceability in Analytical Chemistry	Proficiency testing Quality Assurance
EA	European Cooperation for Accreditation	Accreditation
CEN	European Committee for Normalization	Standardization
IUPAC	International Union of Pure & Applied Chem.	Method validation
ISO	International Standardization Organisation	Standardisation
AOAC	Association of Official Analytical Chemists	Internal qual. Control Proficiency testing
ILAC	International Laboratory Accreditation Cooperat.	Accreditation
FDA	US Food and Drug Administration	Method validation
USP	United States Pharmacopoeia	Method validation
ICH	International Conference on Harmonization	Method validation

A Unique Approach

- **International Conference on Harmonisation (ICH)** was created in 1990
- Agreement between the **EU**, **Japan** and the **USA** to harmonize different regional requirements for registration of pharmaceutical drug products
- Unique because joint effort by regulators and associated pharmaceutical industry trade associations

What is Validation?

- **ICH Guideline Q2(R1) – Text on Validation of Analytical Procedures**

"The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose."

Examples of Methods That Require Validation Documentation

- **Chromatographic Methods - HPLC, GC, TLC, GC/MS, etc.**
  - **Pharmaceutical Analysis**
  - **Bioanalytical Analysis - Clinical Studies.**
- **Spectrophotometric Methods – UV/VIS, IR, NIR, AA, NMR**
- **Capillary Electrophoresis Methods**
- **Particle Size Analysis Methods - Laser, Microscopic**
- **Automated Analytical Methods**

Considerations Prior to Method Validation

- Suitability of Instrument
  - Status of Qualification and Calibration
- Suitability of Materials
  - Status of Reference Standards, Reagents, Placebo Lots
- Suitability of Analyst
  - Status of Training and Qualification Records
- Suitability of Documentation
  - Written analytical procedure and proper approved protocol with pre-established acceptance criteria

Types of Analytical Procedures to be Validated

- Identification tests
- Quantitative tests
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Typical validation characteristics which should be considered

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

ICH Validation Characteristics vs. Type of Analytical Procedure

Type of Analytical Procedure	Identification	Impurity testing		Assay
		Quantitative	Limit Tests	
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Interm. Prec.	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
LOD	No	No	Yes	No
LOQ	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

On the other hand:

Degree to which the assay distinguishes between the target analyte and other components in the sample matrix;

- **the higher the analytical specificity, the lower the level of false-positives.**

Specificity

- **Note:**
  - Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s)
  - Other international authorities (IUPAC, AOAC) have preferred the term "**Selectivity**" reserving "**Specificity**" for those procedures that are completely selective

Specificity

- Some analytical procedures are not sufficiently specific for the intended purpose
  - **Assay by titration**
  - **Assay of enantiomer by achiral method**
  - **Identification by UV absorbance**

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.**

- **A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte.**

Linearity Should be Evaluated

- By Visual Inspection of plot of signals vs. analyte concentration
- By Appropriate statistical methods
  - Linear Regression ($y = mx + b$)
  - Correlation Coefficient, y-intercept (b), slope (m)
- Acceptance criteria: Linear regression $r^2 > 0.95$

Requires a minimum of 5 concentration levels

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.

- The specified range is normally derived from linearity studies and depends on the intended application of the procedure.

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.

- Note:

  This is sometimes termed **trueness**.

Accuracy

Recommended Data

- **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

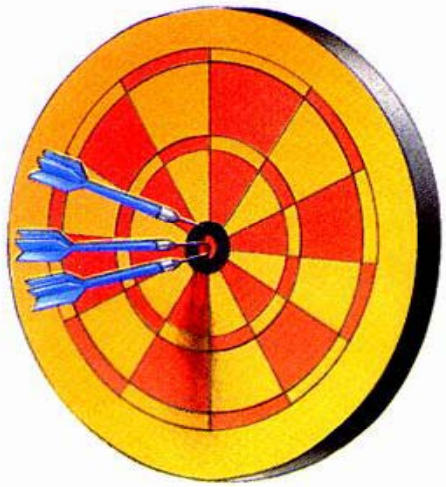
Accuracy Data

Amount Added (mg)	Amount Found (mg)	Percent Recovery
0.0	0.0	---
50.2	50.4	100.5
79.6	80.1	100.6
99.9	100.7	100.8
120.2	119.8	99.7
150.4	149.7	99.5

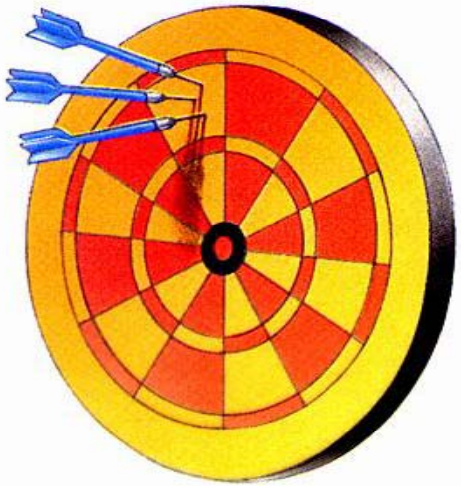
Precision

- 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.

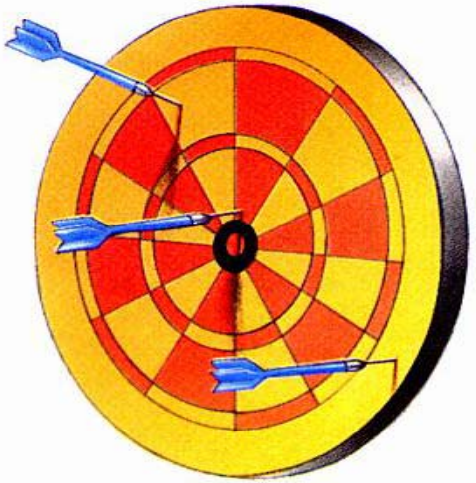
Accuracy v.s Precision



Good accuracy
Good precision



Poor accuracy
Good precision



Poor accuracy
Poor precision

Precision

Precision may be considered at three levels:

- **Repeatability**

  Repeatability expresses the precision under the **same** operating conditions **over a short interval of time**. Repeatability is also termed **intra-assay precision .**

- **Intermediate precision**

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

- **Reproducibility**

  Reproducibility expresses the precision between **laboratories** (collaborative studies, usually applied to standardization of methodology).

Precision

- Precision should be investigated using homogeneous, authentic samples.

- The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability & Intermediate Precision

Day 1	Day 2	Day 2
101	100	98
101	103	102
100	101	98
100	99	97
101	100	101
103	100	102

Mean = 100.9
RSD = 1.5%

Mean = 100.3
RSD = 0.51%

Mean =99.7
RSD = 1.6%

Grand
Mean = 100.3
RSD = 1.63%

Reproducibility

Lab 1		Lab 2		Lab 3	
Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Man 1	Man 2	Man 1	Man 2	Man 1	Man 2
3 Prep	3 Prep	3 Prep	3 Prep	3 Prep	3 Prep

Detection limit (DL)

- The **detection limit** of an individual analytical procedure is the **lowest amount of analyte** in a sample which can be detected but not necessarily quantitated as an exact value.

- Note :
- Detection limit (DL) = Limit of Detection
- Synonymous with " Sensitivity"

Quantitation limit (QL)

- The **quantitation limit** of an individual analytical procedure is **the lowest amount of analyte** in a sample which can be **quantitatively** determined with **suitable precision and accuracy**.

- **Note:**
- Quantitation limit (QL)= Limit of Quantitation (LOQ)

LOD and LOQ Estimated by

1. **Based in Visual Evaluations**

   - Used for non-instrumental methods

2. **Based on Signal-to Noise-Ratio**

   - 3:1 for Detection Limit

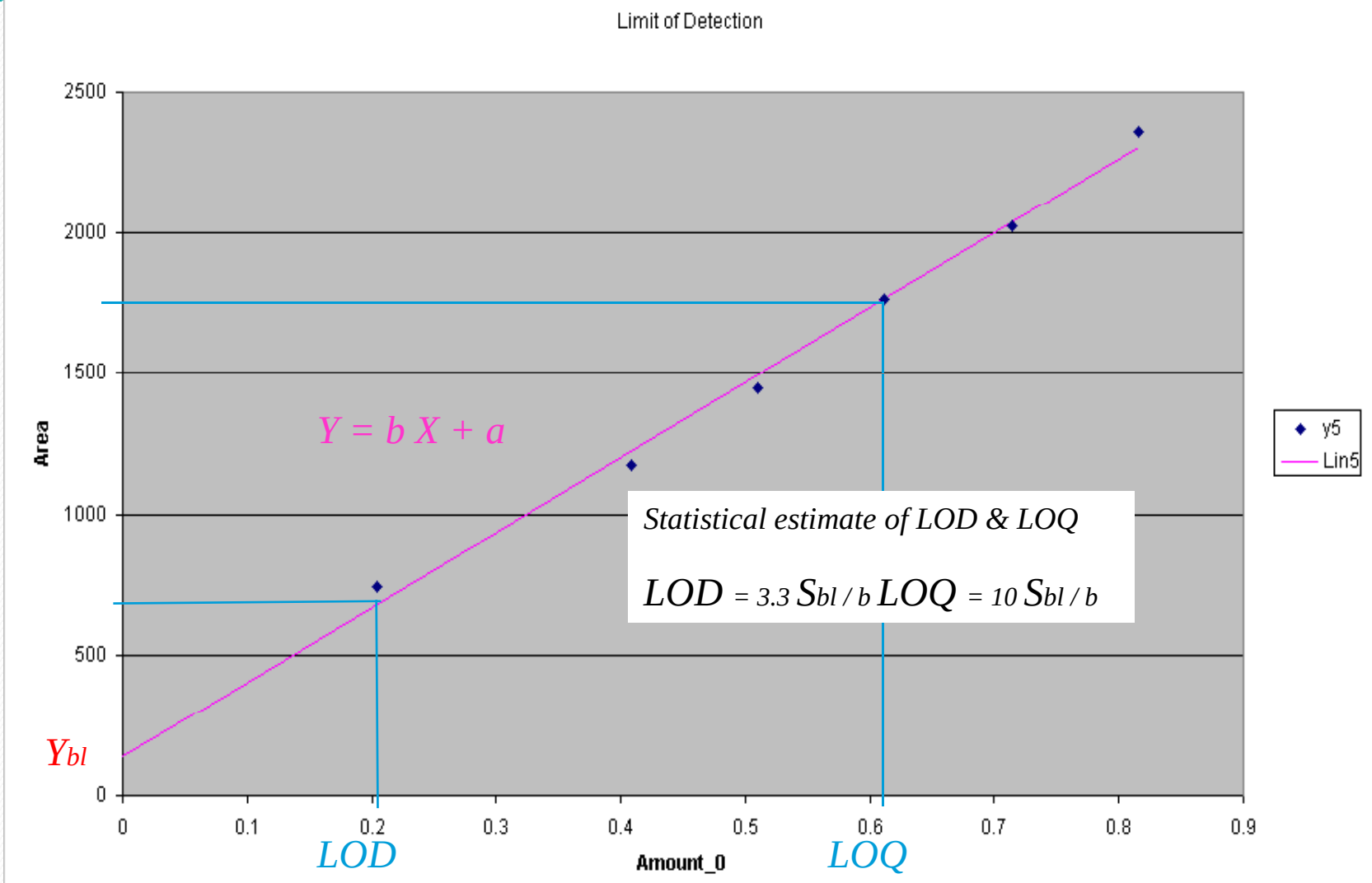
   - 10:1 for Quantitation Limit

3. **Based on Standard Deviation of the Response and the Slope**

LOD and LOQ Estimated by

$$LOD(DL) = \frac{3.3s}{b} \qquad LOQ(QL) = \frac{10s}{b}$$

- **b = slope of calibration curve**
- **s = standard deviation of regression line**


Limit of Detection
2500
2000
1500
1000
500
0
Area
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Amount_0
Y = b X + a
Statistical estimate of LOD & LOQ
LOD = 3.3 Sbl / b LOQ = 10 Sbl / b
Ybl
LOD
LOQ
y5
Lin5

Robustness

- **Definition:** 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.
- **Determination: Comparison results under differing conditions with precision under normal conditions**
- **Examples of typical variations in LC**
  - **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**

Ruggedness

- **Degree of reproducibility of test results under a variety of conditions**
  - **Different Laboratories**
  - **Different Analysts**
  - **Different Instruments**
  - **Different Reagents**
  - **Different Days**
  - **Etc.**
- **Expressed as %RSD**

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