Method validation

- **Method Validation is performed to ensure that an analytical method is accurate, specific, reproducible & rugged over a specified range.**
- **For official method, partial validation is required (accuracy and precision) e.g. Zn SO_4.**
- **For non-official method, total validation is required (all validation parameters) e.g. Fe SO_4.**

- **According to USP. : the EIGHT steps for method validation are:**

✓ Accuracy
✓ Precision
✓ Limit of Detection (LOD)
✓ Limit of Quantification (LOQ)
✓ Specificity
✓ Linearity & Range
✓ Ruggedness
✓ Robustness

- **According to ICH : the NINE steps for method validation are:**

✓ Accuracy
✓ Precision
✓ Limit of Detection (LOD)
✓ Limit of Quantification (LOQ)
✓ Specificity
✓ Linearity
✓ Range
✓ Robustness
✓ System suitability testing

✓ Accuracy:

- It the measure of exactness of an analytical method or the closeness of agreement between the measured value and the value that is accepted either as a conventional, true value or an accepted reference value.
- Expressed by Recovery% **(of RANDOM SAMPLE)**

✓ Precision:

- It is the measure of the degree of repeatability of analytical method.
- Expressed as the percent relative standard deviation for a statistically significant number of samples

✓ Specificity:

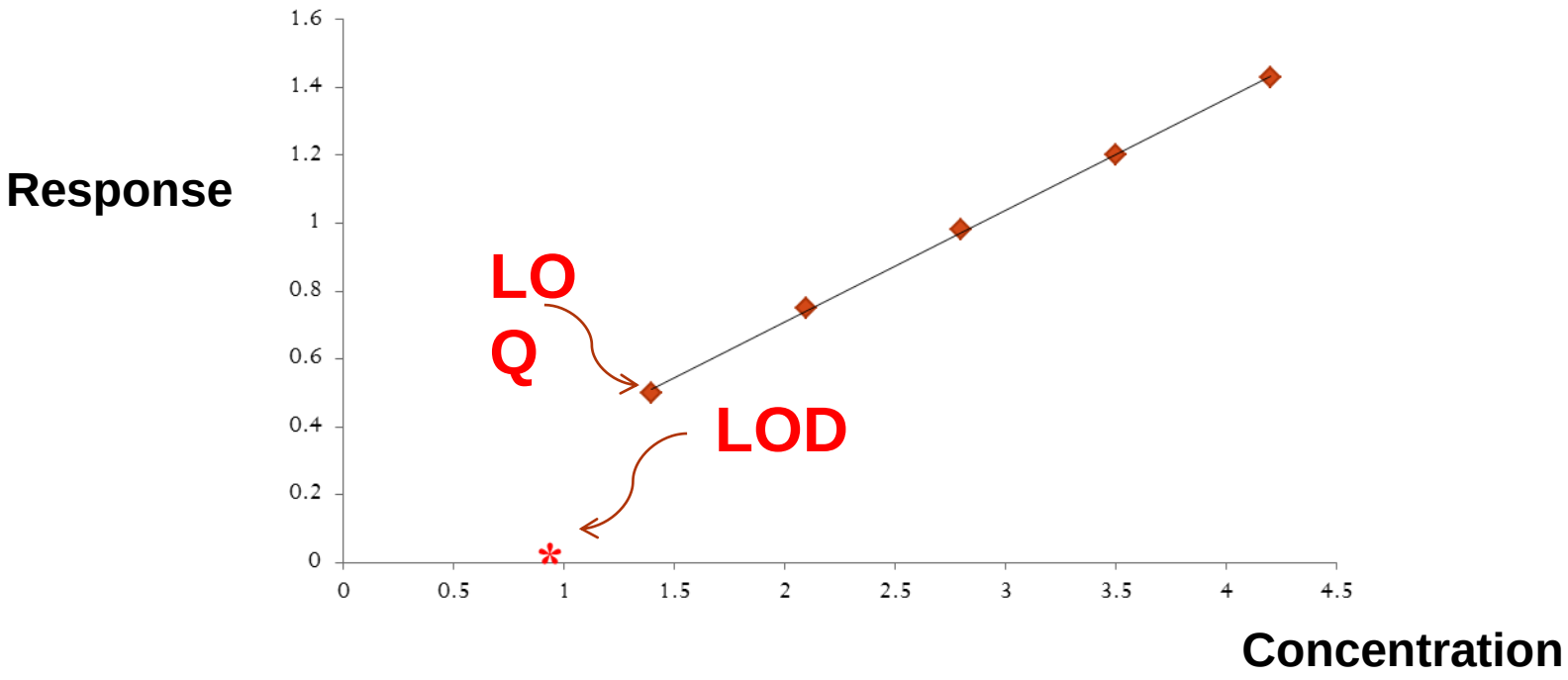
- It is the ability to measure accurately and specifically the analyte of interest in the presence of other components in the sample.

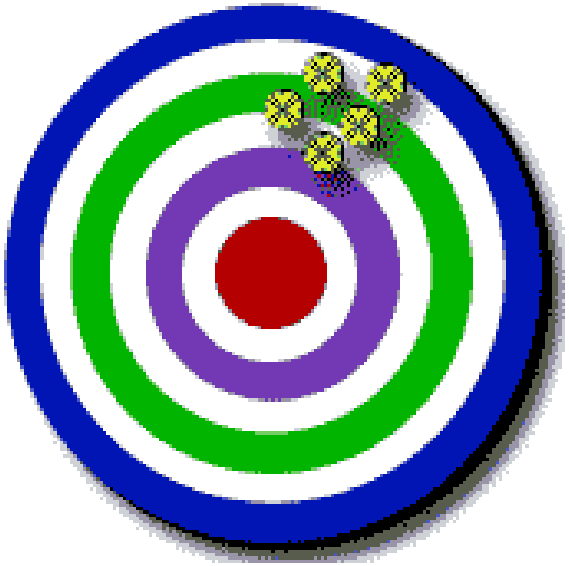
✓ Limit of Detection (LOD):

- Is the lowest concentration of an analyte in a sample that can be detected (NO ACCURACY NOR PRECISION)

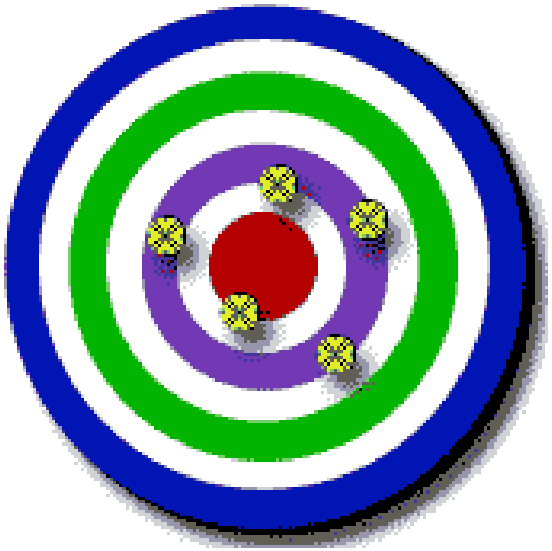
✓ Limit of Quantification (LOQ):

- Is the lowest concentration of an analyte in a sample that can be determined with acceptable precision & accuracy

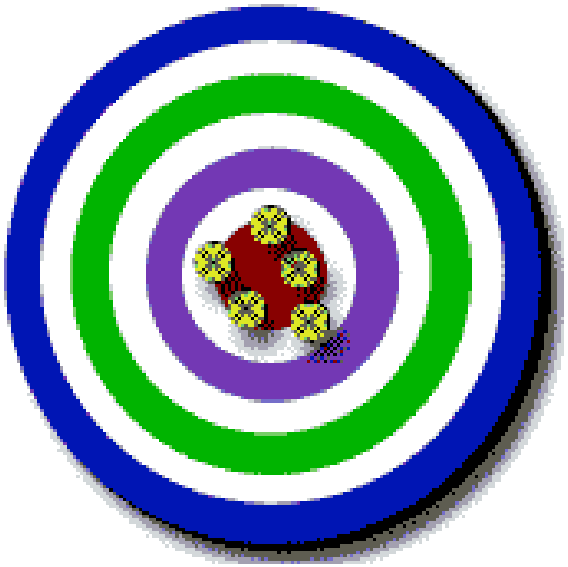




Low accuracy High precision



High accuracy Low precision



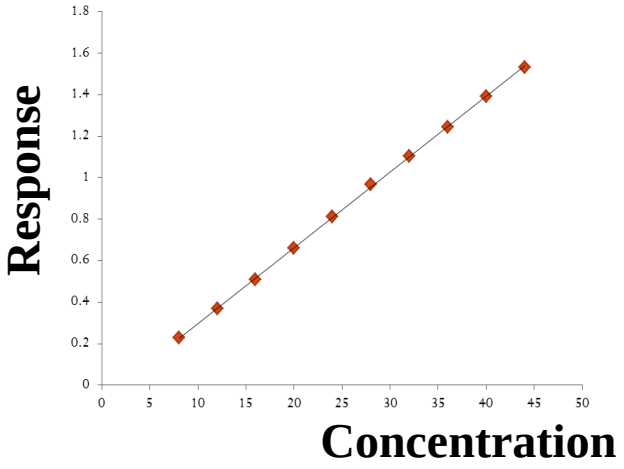
High accuracy High precision

✓Linearity & Range:

Linearity; is the ability of the method to produce test results that are directly proportional to the analyte concentration within a given range.

Indicated by correlation coefficient (r)

Range; is the interval between the *upper & lower* levels (*concentrations*) of analyte that have been demonstrated to be determined with precision, accuracy & linearity.



✓ **Ruggedness:**

- It is the degree of reproducibility of the results obtained under a variety of conditions (as differences in analysts, instruments, reagents & experimental periods)
- Expressed as % relative standard deviation.

✓ **Robustness:**

- It is the capacity of a method to remain unaffected by small deliberate variations in *method parameters (same circumstances)*.

- ✓ **System suitability testing: (added by the ICH "international conference on harmonization")**
- Real samples are always being analyzed before and after the actual batches of samples and often within that run of batches in order to demonstrate that the instrumental system is performing properly before the actual samples are then run

LAB 2

- **Application of colorimetric determination of ferrous sulfate & the assay of commercial product (Ferrofol® capsules)**

- **This lab involves:**
- A) colorimetric method & Construction of calibration curve for ferrous sulfate.
- B) Method validation (by calculating LOD, LOQ, Linearity & range, S.D, R.S.D, precision, accuracy & ruggedness).
- C) Application of the method on Ferrofol® capsules and calculation of found ferrous sulfate amount in capsule.

- **Principle of colorimetric method for determination of ferrous sulfate :**

- **The reduced form of iron (Fe II) can be determined by the reaction with:**

  1,10 phenanthroline (complexing agent or *chromogen*) to form a colored complex in presence of sodium acetate (acts as buffer to stabilize the formed complex)

- **then measure the absorbance of different concentrations of the colored complex.**

A) Colorimetric method & Construction of calibration curve for ferrous sulfate

- **Procedure:**

1) **From a standard solution of ferrous sulfate (2.5×10^{-4} M), take 1, 2, 3, 4 & 5 mL into 5 volumetric flasks (100 mL), respectively.**

2) **Add 3 mL phenanthroline then 2mL sodium acetate to each flask, complete to mark with distilled water and mix well**

3) **Measure the absorbance of each dilution 3 times at 510 nm against blank (in volumetric flask '100ml' put 3 mL phenanthroline and 2mL sodium acetate then complete to mark with distilled water)**

4) **Tabulate the results**

mL	Claimed conc. $g^{\%}*(10^{-4})$ ($2.5x10^{-4}M$ x M.wt.(278) x mL /1000)	A	A’	Found conc.(actual)	Recovery %(found/claimed*100)
1					
2					
3					
4					
5					

5) using EXCEL

- plot a calibration curve between the mean absorbance (A') of each dilution and the concentration in gm %
- Calculate the regression equation of the curve

B) METHOD VALIDATION

6) using the regression equation:

- Calculate the found concentration

7) Calculate the recovery% of each (Recovery % = (found conc. / claimed conc. x 100)

8) Calculate the mean of recovery % , S.D and R.S.D (R.S.D = S.D x 100/ mean of recovery%)

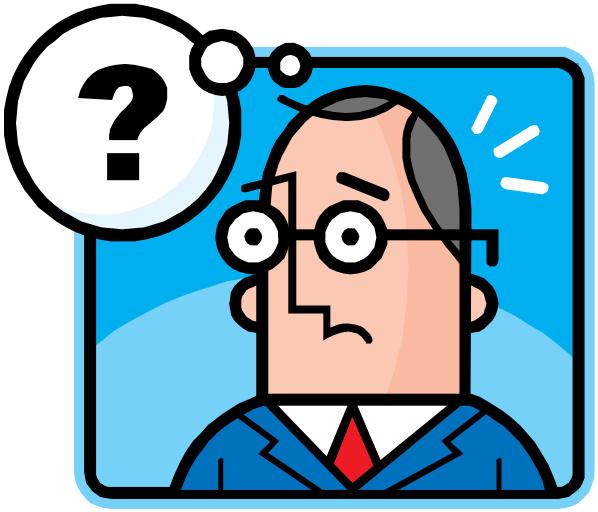
9) From the curve calculate LOD(Given), LOQ and Linearity range

- **10) Determine the ruggedness of method by calculating S.D and R.S.D between measurements of different students** **Ruggedness table**

Analysts	Claimed conc. g%	Absorbance (A)	Average absorbance (A')	Found conc.	Recovery %
1	0.00021				
2	0.00021				
3	0.00021				

- ***11)Accuracy :***
- **If the absorbance of …….g% is ……..**
  Calculate the accuracy%

Accuracy%= [found conc. / labeled conc.] X 100



C) Application of the method on Ferrofol® capsules and calculation of recovery percentage

- **The colorimetric assay of Ferrofol® capsules:**

1) From the **powdered Ferrofol® capsules** , weigh **0.1 gm** then put in 100 mL **volumetric flask**
2) Add **50 ml** distilled **water** & **shake for 5 min**.(to extract $FeSO_4$) complete to the mark with distilled water and **filter**
3) From the extract, transfer **3 mL** into 100 mL **volumetric flask**
4) Add **3 mL of phenanthroline** then **2mL of sodium acetate** and complete to the mark with distilled water
5) Measure the **absorbance** at 510 nm against **blank**
6) Find the **concentration** of the prepared sample using the

Finally

- ✓ **LOD=……**
- ✓ **LOQ = ……**
- ✓ **Accuracy of the given sample= ……**
- ✓ **Linearity range=……**
- ✓ **Regression equation = ……**
- ✓ **S.D of calibration=……**
- ✓ **RSD% of calibration=……**
- ✓ **S.D of Ruggedness =……**
- ✓ **RSD% of Ruggedness=……**
- ✓ **Concentration of Ferrofol® =……**

THANK YOU

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