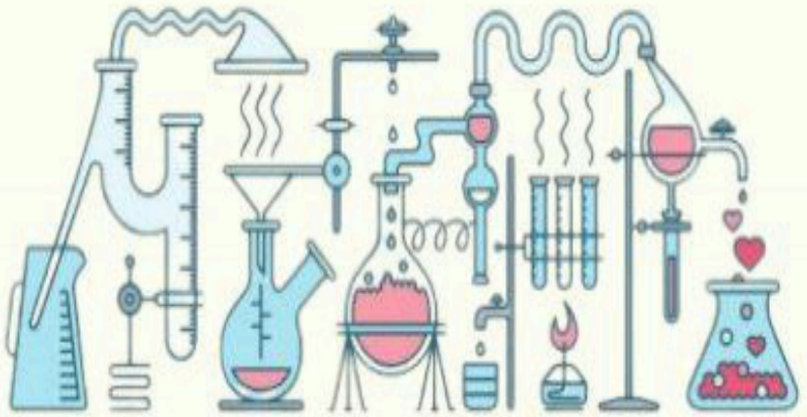
تفريغ تحليل آلي



اسم الموضوع: *Introduction Part 2*

إعداد الصيدلاني/ـة: *Sara Jaber*

Seul Academy

لجان الدفعات

Figures of Merit

عمليات رياضية إذا توافقت الأرقام مع المشروح ☑ Methode Approved

Performance Characteristics: Figures of Merit

How to choose an analytical method? How good is measurement?

or Judge

How well a calibration curve follows a straight line?- **Linearity**.

How reproducible? – **Precision**

How close to true value? - **Accuracy/Bias**

How small a difference can be measured? – **Sensitivity**

What range of amounts? - **Dynamic Range**

How much interference? - **Selectivity**

FIGURES OF MERIT: Linearity

زيادة التركيز مع زيادة الجهاز بشكل متزايد خطي

x → Conc. → ppm
y → Response of Instrument
استجابة الجهاز

- How well a calibration curve follows a straight line.
- R^2 (Square of the correlation coefficient)

$$R^2 = \frac{[\sum(x_i - \bar{x})(y_i - \bar{y})]^2}{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}$$

By calculater

$R^2 > 0.88$ ← Approved ✅

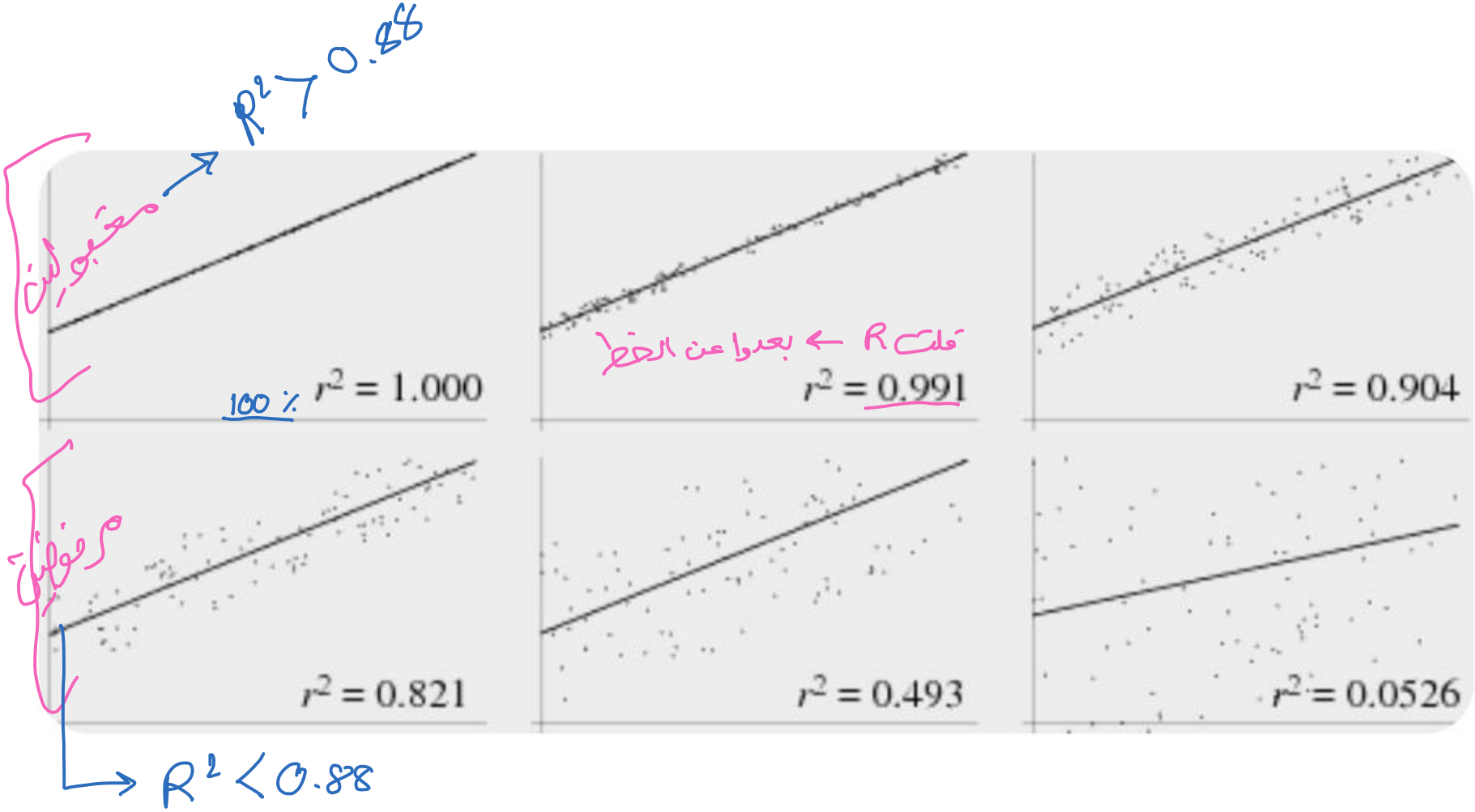
$R^2 < 0.88$ Rejected ❌

[X] → y → Response

1 → ....
2 → ....
5 → ....
10 → ....
25 → ....
50 → ....
100 → ....

$\bar{x}$ = مجموع x / عددهم

$\bar{y}$ = مجموع y / عددهم


$r^2 = 1.000$
$r^2 = 0.991$
$r^2 = 0.904$
$r^2 = 0.821$
$r^2 = 0.493$
$r^2 = 0.0526$

FIGURES OF MERIT: PRECISION = RSD

INDETERMINATE OR RANDOM ERRORS

Standard deviation: $$s = \sqrt{\frac{\sum_{i=0}^{i=N} (x_i - \bar{x})^2}{N-1}}$$

No of x

Variance: s^2

Relative standard deviation: $$RSD = \frac{s}{\bar{x}}$$

دائما مستخدمة

مجهود / عدد x

Standard deviation of mean: $$s_m = \frac{s}{\sqrt{N}}$$

FIGURES OF MERIT: ACCURACY

DETERMINATE ERRORS (operator, method, instrumental)

$$\text{Bias: } \text{bias} = \bar{x} - x_{\text{true}}$$

القانون من صياغة الكتاب والمقصود بـ $\bar{x}$ هو متوسط القراءة التركيز instrumental Responses ⇒ y

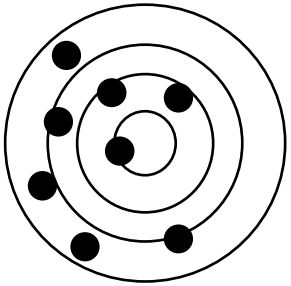
mean – true → "standard" / "Reference" من الشركة

من التجربة ← بكرر التجربة وباخذ القراءات = (y) وبعدها بحسب الـ mean ← $\bar{y}$

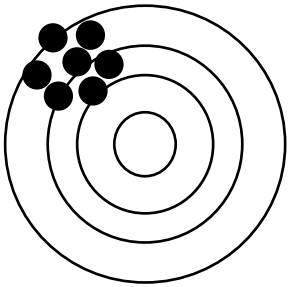
كل ما قل الرقم يعني بقل الفرق بين الـ true value

accuracy ↑ * والعكس *

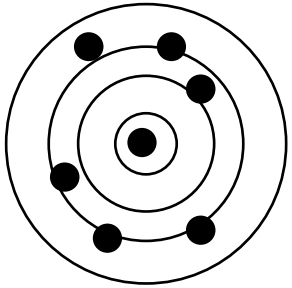
Illustrating the difference between "accuracy" and "precision"



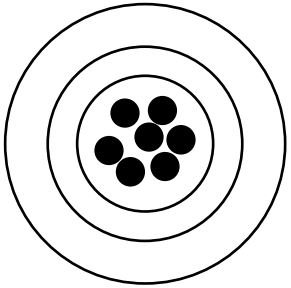
Low accuracy, low precision



Low accuracy, high precision

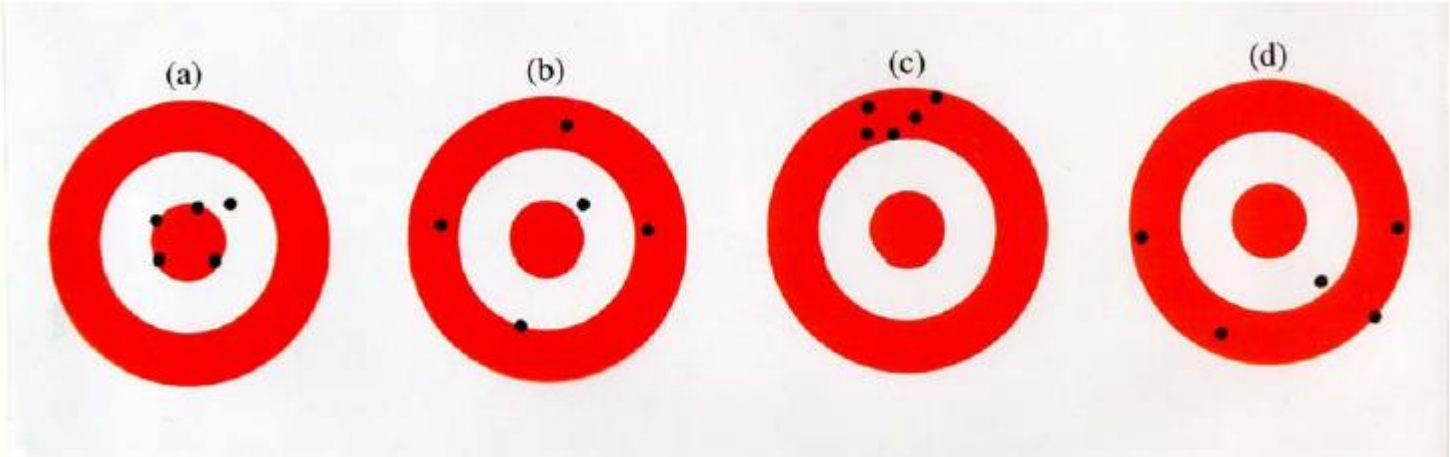


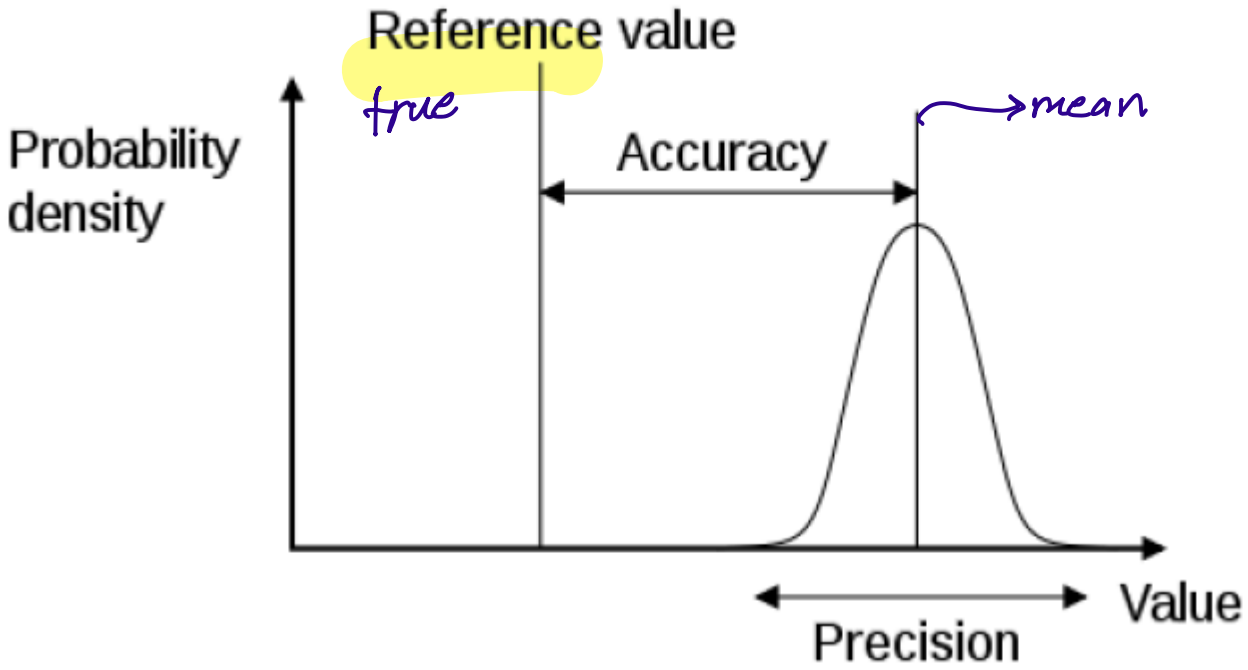
High accuracy, low precision



High accuracy, high precision

Accuracy vs Precision





FIGURES OF MERIT: SENSITIVITY

Method → slope

Method at limit con = $\frac{M}{S_s}$

Indicates the response of the instrument to changes in analyte concentration or a measure of a method's ability to distinguish between small differences in concentration in different samples.

Effected by slope of calibration curve.

Sensitivity = $\gamma = m/S_s$

m = slope;

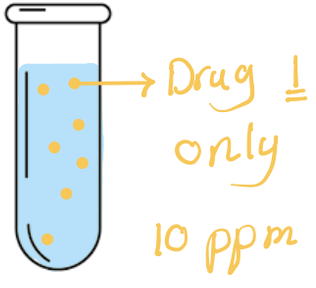
S_s is the standard deviation of measurement

(larger slope of calibration curve m, more sensitive measurement)

قبل الـ LOD, LOQ, LOL في 3 Definitions ←

Stock

فيها تراكيز
مدروسة وانا
اللي بتحكم فيها

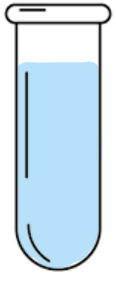


Blank

⇒ فاضية

* لو Sample اللي مطلوب
احللها عبارة عن Urine
⇐ بجيب Urine لشخص
سليم - ما بياخذ دوا.

* لو العينة من نفس ملوث
⇐ بجيب Pure H_2O
يعني Blank من نفس نوع الـ Sample



Blank

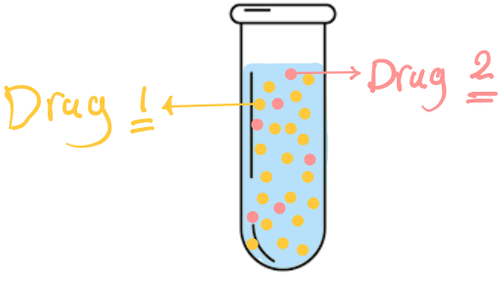
Pure H_2O

Sample

اخذ تحاليل من

- مريض (Blood, Urine)
- مصدر آخر (نهر، بركة، ... Rat Plasma

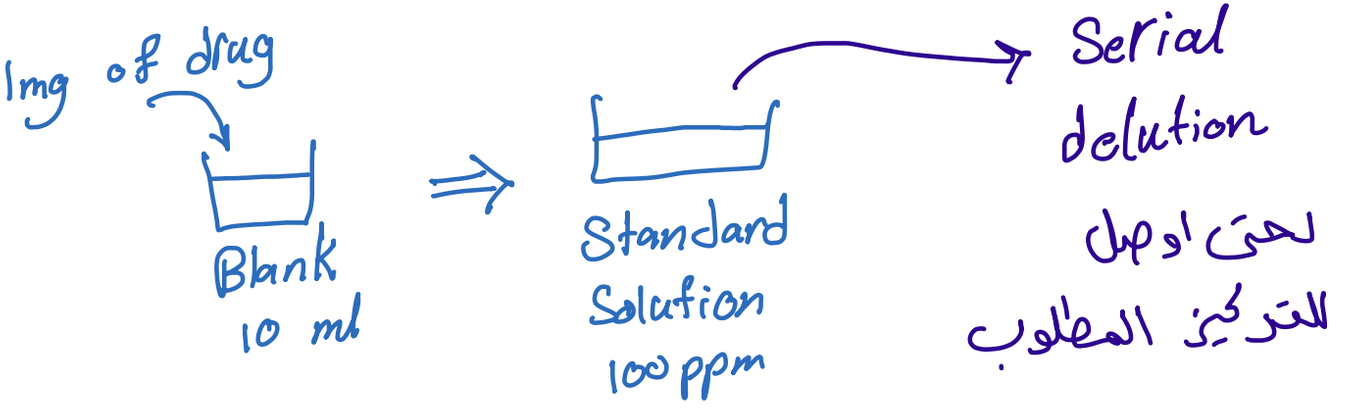
ومطلوب مني اعرف وين المشكلة، بتو الملوث
ونسب الأدوية او الملوثات



بدي اعمل تحليل لـ Urine لمريض ما خذ دواء وعمله مشكل
Sample → drug in urine

Blank → Pure urine without the drug ← لشخص طبيعي

Standard Solution → Blank + تركيز أنا بحطه



FIGURES OF MERIT: LIMIT of DETECTION (LOD)

Signal must be bigger than random noise of blank

Minimum signal: $\text{Signal}_{min} = \text{Av. Signal}_{blank} + k s_{blank}$

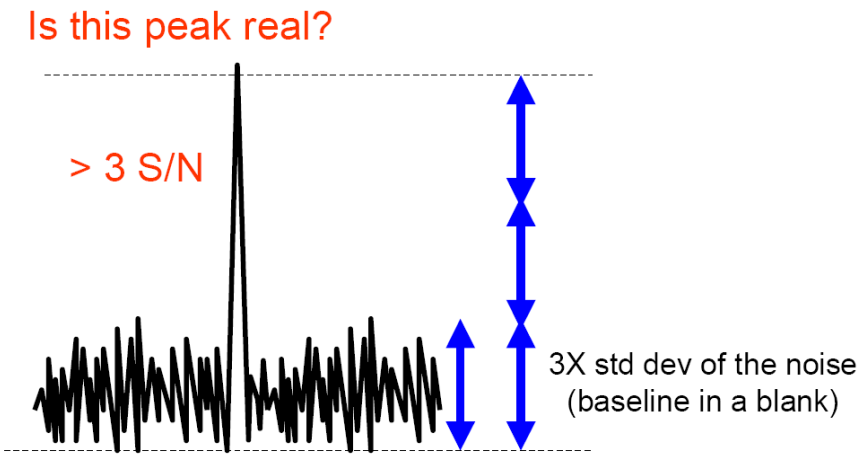
From statistics k=3 or more (at 95% confidence level)

Typically 3 times the signal-to-noise (based on standard deviation of the noise)

or

LOD =3 x Standard deviation of blank/ Slope

LOD=3 $x S_b/m$ → slope



FIGURES OF MERIT: DYNAMIC RANGE

At limit of detection we can say confidently analyte is present but cannot perform reliable quantitation

LOQ (limit of quantification): [lowest] at which quantitative measurements can reliably be made. Equal to 10 x Average Signal for blank *i.e.* 10Sbl. or

LOQ =10 x Standard deviation of blank/ Slope

LOQ=10 xSb/m

Limit of linearity (LOL): when signal is no longer proportional to concentration

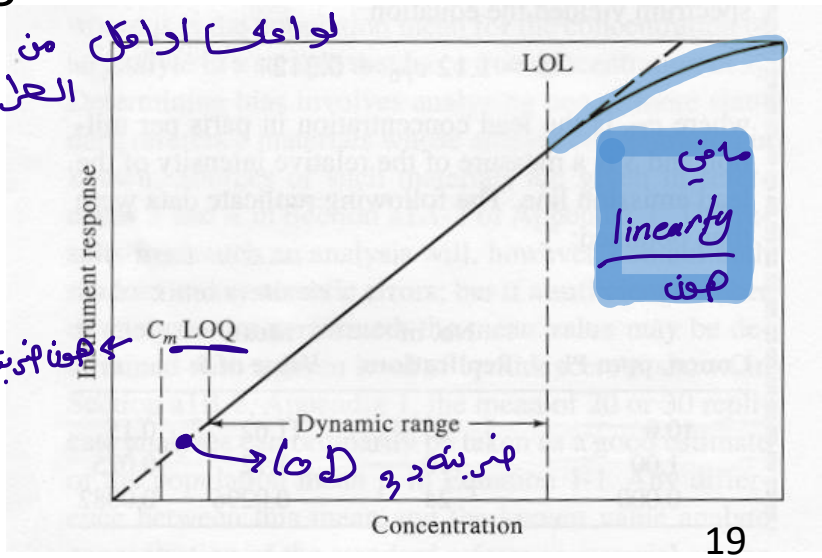
Dynamic range: the maximum range over which an accurate measurement can be made

From limit of quantitation to limit of linearity

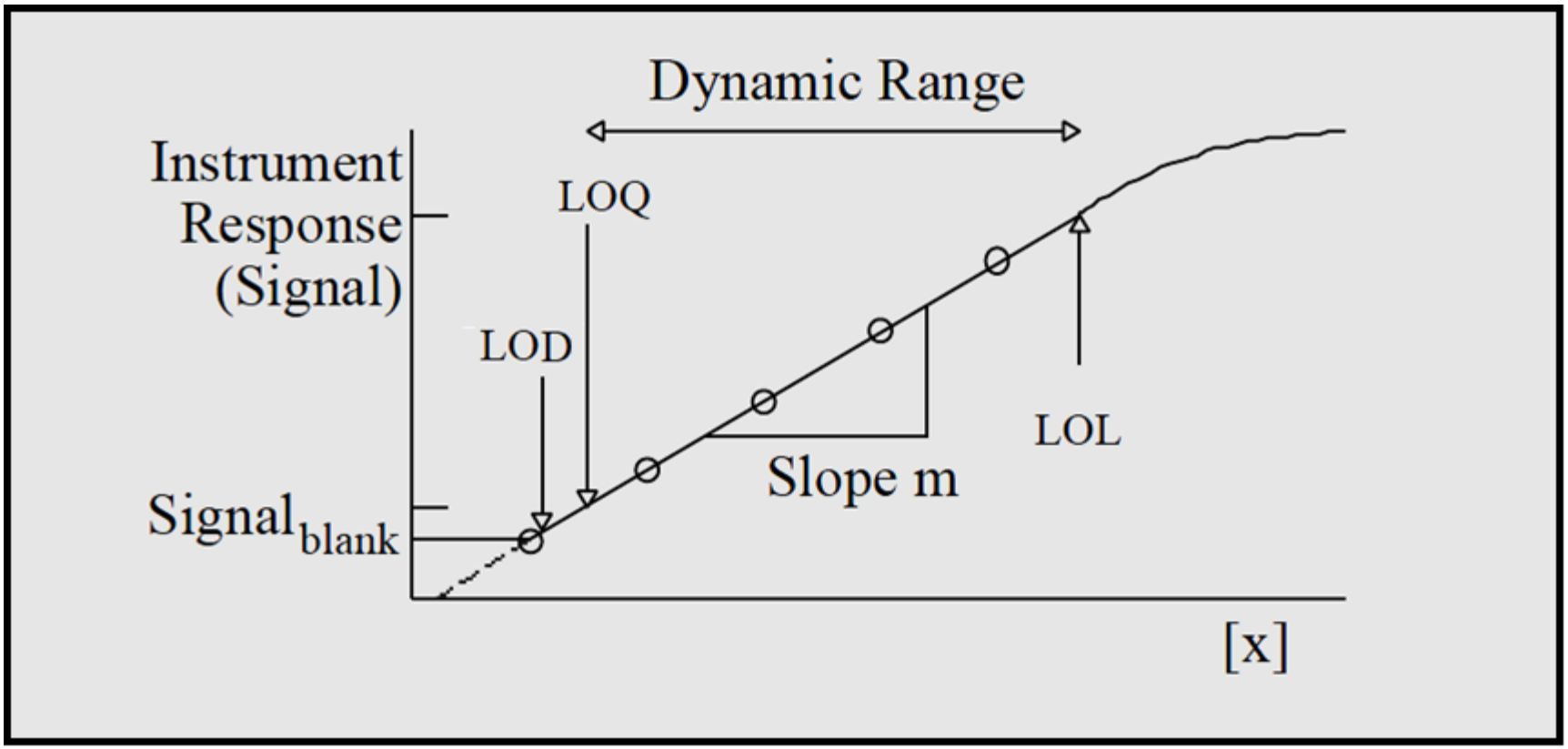
LOQ: 10 s of blank

LOL: 5% deviation from linear

$$\text{Dynamic range: } \frac{\text{LOL}}{\text{LOQ}} \qquad 10^2 \text{ to } > 10^6$$



CALIBRATION CURVES (WORKING or ANALYTICAL)



عشان تعملي مع تسلسل الشرح

CALIBRATION METHODS

Basis of *quantitative* analysis is magnitude of *measured property* is proportional to *concentration* of analyte

$y = \text{Slope } X \pm \text{intercept}$

اول اشي بعمله بطلع الـ calibration eqn.

$$\text{Signal} \propto [x] \quad \text{or} \quad \text{Signal} = m[x] + \text{Signal}_{\text{blank}}$$

y slope conc

$$[x] = \frac{\text{Signal} - \text{Signal}_{\text{blank}}}{m}$$

بجزء المختبر العملي

Salt + Water → Stock

Blank

بعمل اكثر من Solution و بدخلهم عـ الجهاز وباخذ القراءة

SAMPLE PROBLEM:

اول اشي نلازم اطلع الـ equ
بدي m
intercept

Analyte Concentration (ppm*)	Absorbance
0.0 (blank) صافي اشي	0.05
0.9	0.15
2.0	0.24
3.1	0.33
4.1	0.42

1- Slope $= \frac{S_{xy}}{S_{xx}}$

Define Variance and Covariance:

$$S_{xx} = \frac{\sum (x_i - \bar{x})^2}{N-1} \qquad S_{xy} = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{N-1}$$

$$\bar{x} = 2.02 \quad \bar{y} = 0.238$$

$$S_{xx} = \frac{\left(2.02^2 + 1.12^2 + 0.02^2 + 1.08^2 + 2.08^2\right)}{4} = \frac{10.828}{4} = 2.707$$

$$S_{xy} = \frac{(-2.02 \times -0.188) + (-1.12 \times -0.088) + (-0.02 \times 0.002) + \ldots}{4}$$

$$= \frac{0.9562}{4} = 0.23905$$

2- intercept $= \bar{y} - m\bar{x}$

Slope: $m = \frac{S_{xy}}{Sx} = \frac{0.23905}{2.707} = 0.0883$

$b = \bar{y} - m\bar{x}$

Intercept: $= 0.238 - (0.0883 \times 2.02)$

$= 0.0596$ ← لو كانت سالبة بنزلها سالبة

Calibration expression is

Absorbance=0.0883[Analyte (ppm)]+0.0596

$y = 0.088x + 0.0596$

جيب شو بستفيد من المعادلة؟

- بجيب الـ Sample وبحطها بالجهاز وبطلع قراءة y

ومن المعادلة بوجد x ← conc

Example:

An analysis of the calibration data for the determination of lead based upon its flame emission spectrum yielded an equation: $S = 1.12C + 0.312$ where C is the Pb concn in ppm and S is a measure of the relative emission intensity. The following replicate data were obtained:

$y = 1.12x + 0.312$ $b = 0.312$ $m = 1.12$

Concn (C), ppm	No. of replicates	Mean value of S	Std. dev.
10.0	10	11.62	0.15
1.00	10	1.12	0.025
0.000	24	0.0296	0.0082

Blank

Calculate (a) the calibration sensitivity, (b) the analytical sensitivity at 1 and 10 ppm of Pb,(c) the limit of detection limit (LOD), and ,(d) the limit of quantification limit (LOQ)

Example Solution:

a) calibration sensitivity is the slope of the calibration curve = 1.12

b) Using $\gamma = m/S_s$: at 10 ppm $\gamma = 1.12 / 0.15 = 7.5$ at 1 ppm

$\gamma = 1.12 / 0.025 = 45$

c) LOD= 3 x Sb/ slope= 3 x 0.0082/1.12 = 0.0219

d) LOQ= 10 x Sb/ slope= 10 x 0.0082/1.12 = 0.0732