



# **PHARMACEUTICAL INSTRUMENTAL ANALYSIS**

**Dr Aref Zayed**

identify dose → 500mg  
بدون دوز → 5mg  
نشان می‌دهد  
more precise / accurate → toxic.  
analysis dose → quantitative.  
- qualitative.

# COURSE OUTLINE

## 1. Introduction to Instrumental Methods of Analysis.

## 2. Spectrometry:

- Introduction to Electromagnetic Radiation
- Quantum-Mechanical Properties of Radiation.

} (in)

## 3. Ultra-Violet (UV) & Visible Spectrometry

## 4. Infrared Spectroscopy (IR).

## 5. Nuclear Magnetic Resonance (NMR) Spectroscopy : Uses in Identification of Organic Compounds

## 6. Mass Spectrometry (MS): Uses in Quantitative and Qualitative

## 7. Chromatography:

1. Liquid Chromatography (LC)
2. Gas Chromatography (GC).

\* First  
الكل





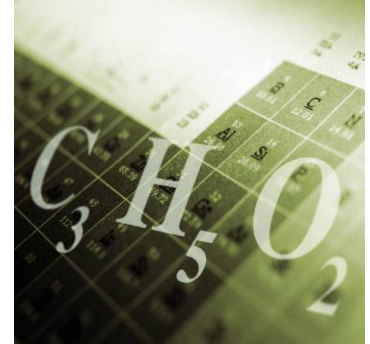
# **PHARMACEUTICAL INSTRUMENTAL ANALYSIS**

## **Selecting an Analytical Method**

# INTRODUCTION

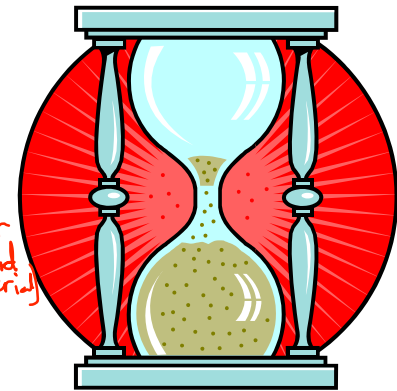
## Types of Analyses:

1. Quantitative → *مقاييس* Conc. / Percentage
  2. Qualitative → structure.  
 → MWT  
 → functional group
- Handwritten notes for Qualitative:*  
 - نوع المادة / المودا  
 - التركيب الكيميائي  
 - main functional group  
 - كيفية صيغته  
 - كيفية تفاعل المادة المحلولة



## Classification of Analytical Methods (Historical):

1. Classical → titration → Find Conc. acid or base. → indicator  
 ↓  
 indicator (coloured material)
  2. Instrumental → *تقني*  
 - colour  
 - taste  
 - smell  
 - absorption  
 - emission  
 - resistance  
 - Br2
- Handwritten notes for Instrumental:*  
 - تفاعل  
 - اصلاصة بالعين  
 - المجرده على مياسه



# CLASSIFICATION OF ANALYTICAL METHODS

## 1. Classical Methods:

- Analytes **separated** by precipitation, extraction or distillation.
- If **Qualitative**: Analytes recognised by Colour, B.P, M.P, Solubility, Odour, Refractive Index.
- If **Quantitative**: Amount determined by Gravimetric or Titrimetric measurement.

## 2. Instrumental Methods:

- Reliable and modern instruments were introduced.
- Measurement of physical properties like conductivity, electrode potential, mass to charge ratio, light absorption/ emission.

- ✓ • Highly efficient separation techniques.  
Chromatography, Electrophoresis.

→  
✓  
technique to separate things

classical →  
→ separation  
→ participation  
- extraction.

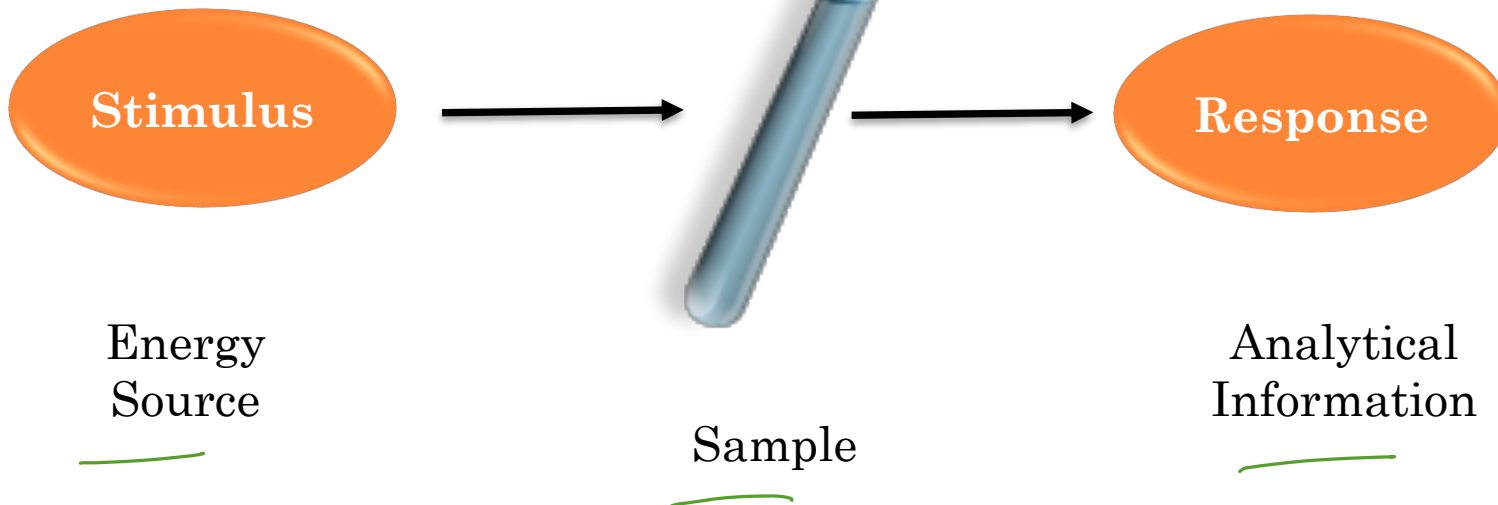
# INSTRUMENTS FOR ANALYSIS

تحليل  
هي الملاحظة المراد فحصها

An **instrument** for chemical analysis converts information stored in the physical or chemical characteristics of the analyte to information that can be manipulated and interpreted by a **human**.

لا أستطيع  
رؤيتها  
→ translate  
to informant  
I can read it

سكر → خلوص → لا أعلن في  
خلوص → العلم بالعرف  
المعتمدة  
→ مبررة  
ال  
للتنوع  
تلك  
صية فاصم يمين  
بعلوه لا أعلن ان  
على غللا و الجول  
الى قرادة صبره  
استطيع فهمها



# TYPES OF INSTRUMENTAL METHODS

صنوع ← موجات ، مبني  
الكلاطية مزدوجة

- According to characteristic properties employed to generate analytical signal:

صنوع اذا دخل على  
مادة يتغير بخاصة  
و اذا اخرج يغير على شكل موجات  
صن مترجم

types  
1 depend  
light

Instrument technique built in those which depends on electromagnetic\* radiation.

## Properties involving Electromagnetic Radiation:

Xray, NMR

- Absorption, emission, scattering...

e.g. UV, IR, NMR, fluorescence, phosphorescence

interaction between source of radiation & molecules

\* مبني على اجزئة الجسيم

- UV → molecules → electronic transition\*  
IR → — → vibration & rotation  
NMR → nuclear → spin.

## 2. Electrical Properties:

electrode  
كنا د +

Electrical potential, charge, current or resistance.

## 3. Miscellaneous:

- Mass-to-charge ratio, mass, radioactivity.

e.g. Mass Spectrometry (MS)

قياس  
الكتلة الجزيئية (Mwt.)



# ANALYTICAL METHOD SELECTION

كل صفة لنفس العوزن  
هنا صفة صفة حصة من  
19.

How we select the method:-

- characteristics of the problem

Sample size or small  
تركيز عالي او قليل

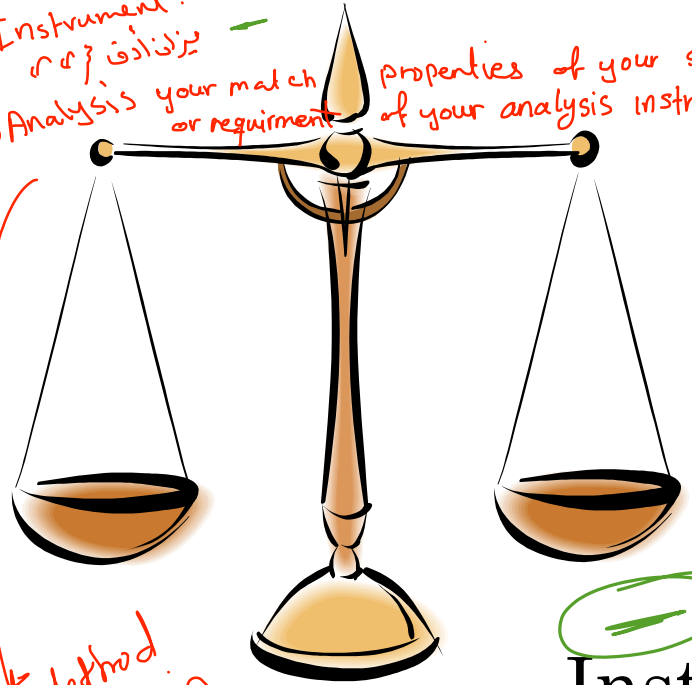
- the type of compound I can use to detect

- after characterize your sample.

Instrument

Analysis your match or requirement

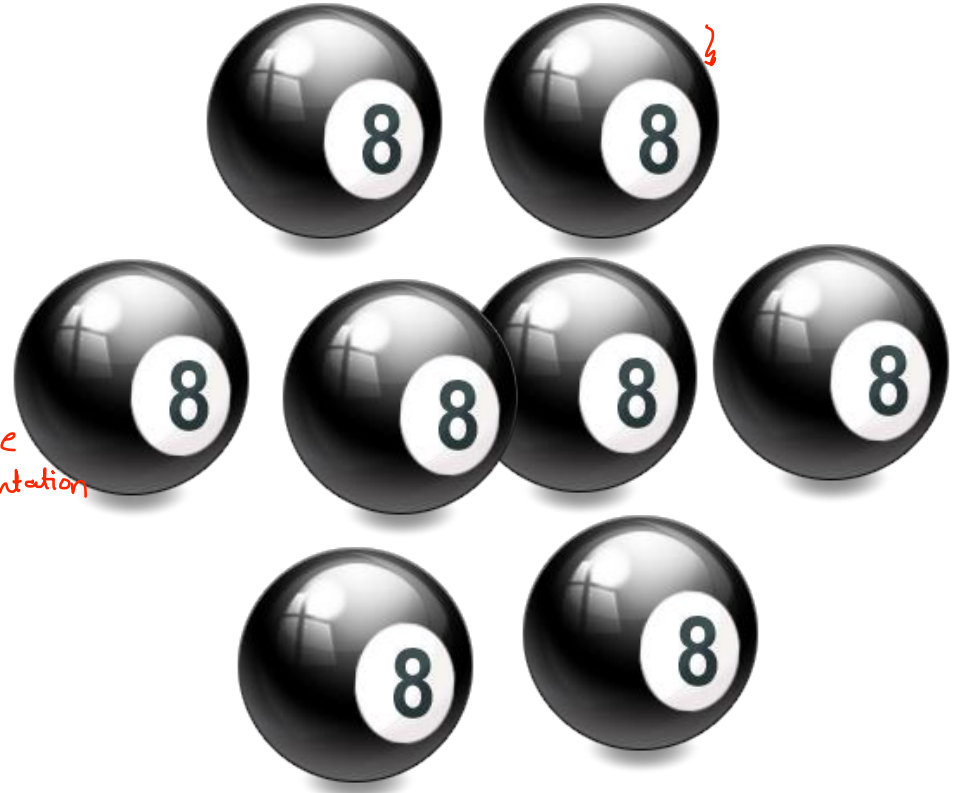
properties of your sample of your analysis instrumentation



Method selection



Instrument



Sample



## 1E SELECTING AN ANALYTICAL METHOD

Column 2 of Table 1-1 shows that today we have an enormous array of tools for performing chemical analyses. There are so many, in fact, that the choice among them is often difficult. In this section, we briefly describe how such choices are made.

### 1E-1 Defining the Problem

To select an analytical method intelligently, it is essential to define clearly the nature of the analytical problem. Such a definition requires answers to the following questions:

1. What accuracy is required? —
2. How much sample is available? ⊕
3. What is the concentration range of the analyte? [- +]
4. What components of the sample might cause interference? ⊕
5. What are the physical and chemical properties of the sample matrix? ⊕
6. How many samples are to be analyzed?

The answer to question 1 is of vital importance because it determines how much time and care will be needed for the analysis. The answers to questions 2 and 3 determine how sensitive the

method must be and how wide a range of concentrations must be accommodated. The answer to question 4 determines the selectivity required of the method. The answers to question 5 are important because some analytical methods in Table 1-1 are applicable to solutions (usually aqueous) of the analyte. Other methods are more easily applied to gaseous samples, and still other methods are suited to the direct analysis of solids.

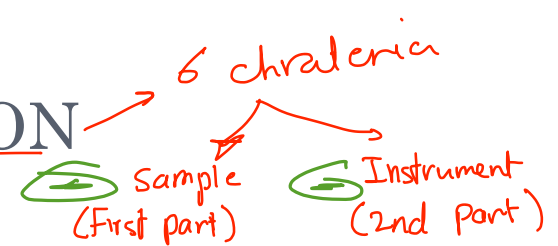
The number of samples to be analyzed (question 6) is also an important consideration from an economic standpoint. If this number is large, considerable time and money can be spent on instrumentation, method development, and calibration. Furthermore, if the number is large, a method should be chosen that requires the least operator time per sample. On the other hand, if only a few samples are to be analyzed, a simpler but more time-consuming method that requires little or no preliminary work is often the wiser choice.

With answers to these six questions, a method can then be chosen, provided that the performance characteristics of the various instruments shown in Table 1-1 are known.

### 1E-2 Performance Characteristics of Instruments

Table 1-3 lists quantitative instrument performance criteria that can be used to decide whether a given instrumental method is suitable for attacking an analytical problem.

# ANALYTICAL METHOD SELECTION



○ Define the problem: How you can characterize your sample.

1. Accuracy (e.g. need 2-figure or 5-figure balance?)

is the degree of agreement between the measured value & the true value / absolute error =  $|X_i - X_t|$  / relative error =  $\frac{|X_i - X_t|}{X_t} \times 100\%$

2. Amount / concentration of sample e.g. measure drug concentration in blood

- 2mg صندل  
- Amoxicillin مضاد حيوي 625mg / 6L blood → does accepted. انقبض

3. Concentration Range

Instrument able to measure from lowest conc. to highest conc.

4. Interferences (e.g. Melamine contamination)

signal (Ex: -)

حادة نسبت بينهم في تحليل الكمال المصنع  
protein → N → Melamine → Interference.

تباين بوجود بالعينة  
نتج sample لكن لا نتاجر وكفوت علينا  
(vitamin, lipid, protein)

5. Matrix physical and chemical properties

Environment كبرهم  
blood يتغير → water.

6. Number of samples (cost, time, automation)



\* Instrument.

# ANALYTICAL METHOD SELECTION

## Instrument Performance Characteristics:

### 1. Precision:

- Agreement among data obtained in same way (replicate measurements) درجة تقارب العينات من بعضها البعض لنفس العينة.
- Measure of random errors

e.g. Measure glucose levels in a blood sample (3 replicates) by two methods:

Method A: 70, 80 and 90 mg/dL

Method B: 78, 80, and 82 mg/dl

Which method is more precise?

Avg  $\frac{80}{3}$   
X

طريقة الـ 80  
الأغزاف المعيارية



# ANALYTICAL METHOD SELECTION

- Precision can be expressed by calculating standard deviation

we need Low(S) → to say our method is precise

حفظ

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}} \rightarrow \frac{\sum x}{n}$$

$$RSD = \frac{SD}{\bar{x}} \times 100\% \quad \text{or Coefficient of variation}$$

- For method A in the previous example :

- $\bar{x} = (70+80+90)/3 = 80$
- $(x-\bar{x}) = 70-80, 80-80, 90-80 = -10, 0, 10$
- $(x-\bar{x})^2 = 100, 0, 100$
- $\sum(x-\bar{x})^2 = 100+0+100 = 200$

- $s = \sqrt{\frac{200}{3-1}} = 10$

Method B

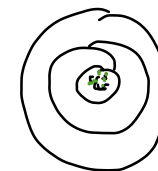
x	x - $\bar{x}$
70	(-10) <sup>2</sup>
80	(0) <sup>2</sup>
90	(10) <sup>2</sup>
$\bar{x} = 80$	$\sum(x-\bar{x})^2$

precision

كلما كانت قيمة RSD قليلة تكون  
precision عالية



high precise  
Low accuracy



high precise ✓  
high accuracy

# ANALYTICAL METHOD SELECTION

See  
Incl.

## Instrument Performance Characteristics

2. **Bias**: a measure of systematic error

(Instrument)

system: یزانی

accuracy  
↓  
error  
See 1

← نمرات قراءات اکثر من جزء دلظوری

True Value

$$\text{bias} = \mu - x_t$$

Mean value

True value

Accuracy (sample)



# USEFUL TERMS



○ **Blank** : is a sample that contains all of the components of the original sample except for the analyte.

(عينة لهفرية) ستي خاض

عينة خلوة ← عينة blank عبارة عن حبة له اطا لا تحتوي على علوكوز.

○ **Calibration curve**: is used by introducing several standards containing exactly known concentrations of the analyte into the instrument.

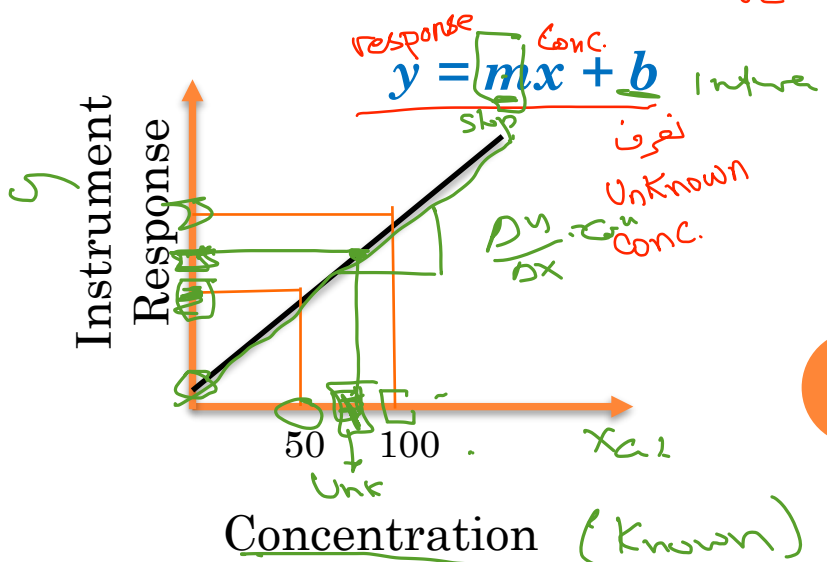
المادة المراد تحليلها

quantitative analysis.

↓  
need Calibration Curve.

HPK  
Self  
ليان مطوية

Concentration	Instrument Response
0	10
50	45
100	90
Unknown Conc	75



# ANALYTICAL METHOD SELECTION

## Instrument Performance Characteristics

slope  
LOD3 > **Sensitivity** أي تغيير بالتركيز  
لعينه تغيير بالقراد

- Ability of method to distinguish between small differences in analyte concentration.

**Depends on :**

- Slope of calibration curve
- Precision (reproducibility)

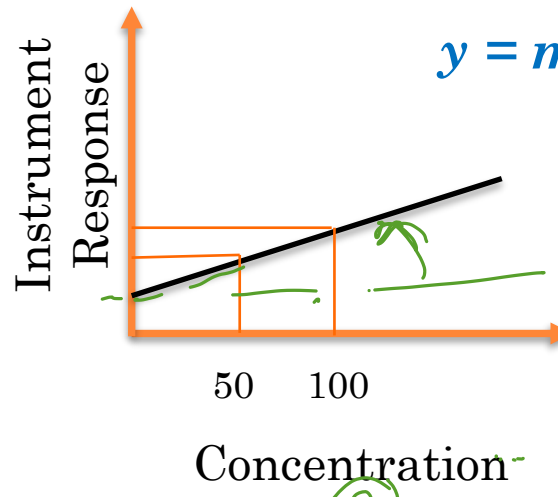
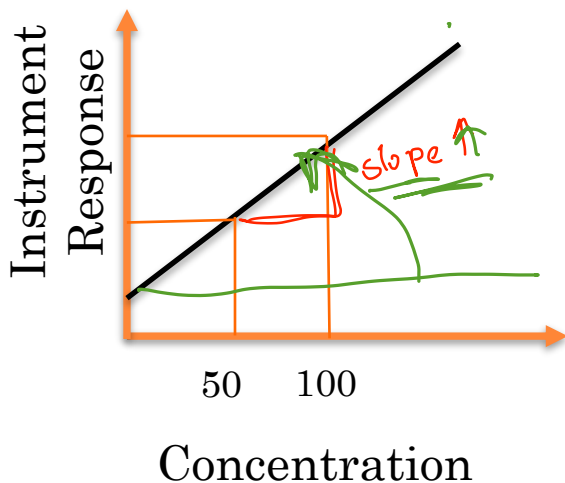
calibration sensitivity is independent of the concentration

$$Y = \frac{m}{SD} = \frac{\text{slope}}{\text{standard deviation}}$$

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

2 calibration curve in 2 instrument.

Which method is more sensitive?



$$y = mx + b$$

slope =  $\frac{y}{x}$

$$y \propto m$$



# ANALYTICAL METHOD SELECTION

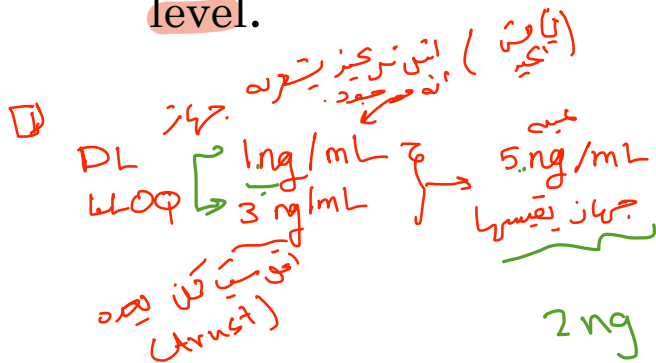
## Instrument Performance Characteristics

4. Detection limit (DL): The minimum concentration or mass of the analyte the can be detected at a known confidence level.

by instruments  
Detect is not quantified.

Lower Limit of Quantification (LLOQ): The minimum concentration or mass of the analyte the can be quantified at a known confidence level.

] not mentioned



$$DL = \frac{3 sB}{m}$$

Standard deviation of the blank

Calibration curve slope

$$LLOQ = \frac{10 sB}{m}$$

→ 2ng/mL ALcho / blood.  
→ > DL Yes, there is this analyte  
لكن ما هو التركيز هل هو 2 - لا دلتا موجود  
فقط 3ng هنا تكون متأكدين من وجوده ومن تعريف

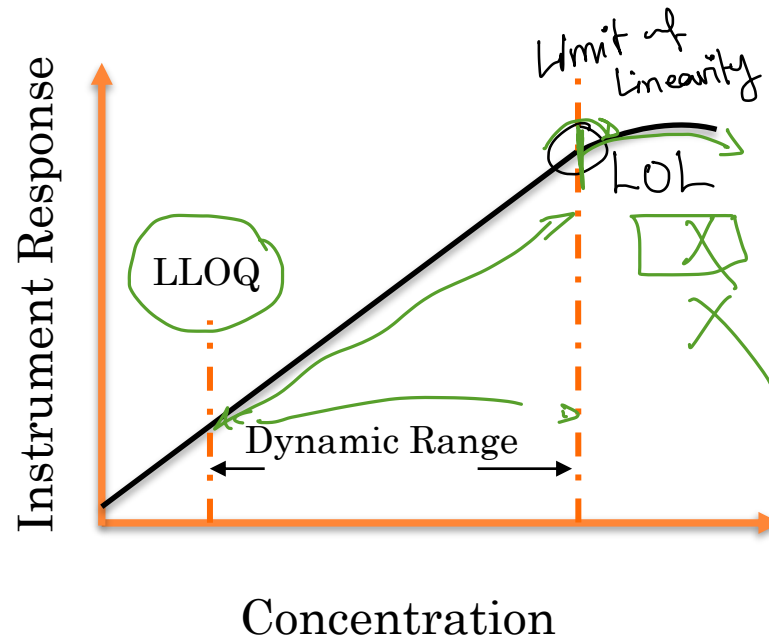
سلاطات (Sensitivity) LLOQ أقل [علاقة طرسيه]  
Sensitivity and DL? → 0.05 ng/mL < DL  
متى نغطي حكم هل هو موجود analyte  
ما لا يجب ان نستخدم جهاز له حساسية جواز اعلى من المستخدم

# ANALYTICAL METHOD SELECTION

جهاز الذي سوف استخدمه له  
rang اقل  
rang اتى

## Instrument Performance Characteristics

- Dynamic Range:** It is the range that extends from the lowest concentration at which quantitative measurements can be made (LLOQ) to the concentration at which the calibration curve departs from linearity.



إذا حدث الانحراف عن  
النقطة المستقيمة  
dynamic range  
لا يحقّ أن تكون  
accuracy + precision



# ANALYTICAL METHOD SELECTION

## ○ Instrument Performance Characteristics

(sample) Interference  
↑  
(analyte)

مداخله در  
Interferences.

6. Selectivity: The degree to which the analytical method is free from interference by other species contained in the sample matrix.

select  
↓  
مداخله  
↓



# ANALYTICAL METHOD SELECTION

## Instrument Performance Characteristics

- Other characteristics for selecting analytical methods:

6. Speed → The time can be important if the result time contributes to the cost.

are required urgently  
(اختيار سرعة وتكلفة)  
الوقت، كما يبدأ على  
الوقت الذي تزيده بالوقت  
المطلوب

7. Ease and convenience.

8. Skill required of operator. ✓

9. Cost and availability of equipment.

10. Per-sample cost. [preparation]

من حيث  
تكلفة التحليل

(reagents, equipment / staffing cost)

\* Safety → is so important parameter.

\* Automation →  
accuracy  
precision  
دلائل ان نتائج  
البيانات تزيده على

→ reduce operator time, reproducibility



# NOTES

## ○ Calibration of Instrumental Methods: (self study from the book)

*2 points in Exam.*

*5 paper in book.*

- Calibration curves ✓
- Standard addition methods ✓
- Internal standard method ✓

*new video*  
*↑*



# ANALYTICAL PROCESS

— لغرض التعرف وما هي  
— كيف نقوم بجمعها

— كمرضى مع ناخذ دم  
— عينات خسوا بلة

Sampling

- Obtain a **representative** bulk sample from the lot.
- Obtain a **homogenous** laboratory sample from the bulk sample

Sample Preparation

- Convert sample into a form suitable for analysis.
- **Remove species that interfere with chemical analysis**
- e.g: Sample **pre-concentration**, **protein precipitation**, **liquid-liquid extraction**, **filtration**, **solid phase extraction**

↓ centrifuge  
طريقة فعالة  
لقلع matrix  
دون ان ستأثر العينة

Analysis

- Run standards and samples.  
طريقة الجهاز المناسب

— Unknown  
— blank.  
— standard calibration

Results

- Record and evaluate results of the analysis

فهموه  
الشيء  
الذي طلب نتائج

Report and Evaluation

