Pharmaceutical Instrumental Analysis

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Course Outline

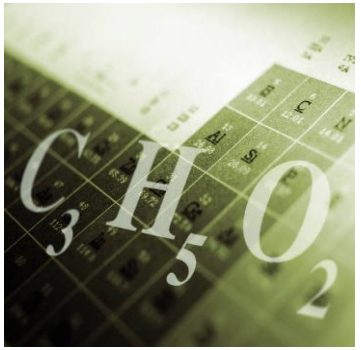
1. Introduction to Instrumental Methods of Analysis.
2. Spectrometry:
   - Introduction to Electromagnetic Radiation
   - Quantum-Mechanical Properties of Radiation.
3. Ultra-Violet (UV) & Visible Spectrometry
4. Infrared Spectroscopy (IR).
5. Nuclear Magnetic Resonance (NMR) Spectroscopy : Uses in Identification of Organic Compounds
6. Mass Spectrometry (MS): Uses in Quantitative and Qualitative
7. Chromatography:
   1. Liquid Chromatography (LC)
   2. Gas Chromatography (GC).

Pharmaceutical Instrumental Analysis

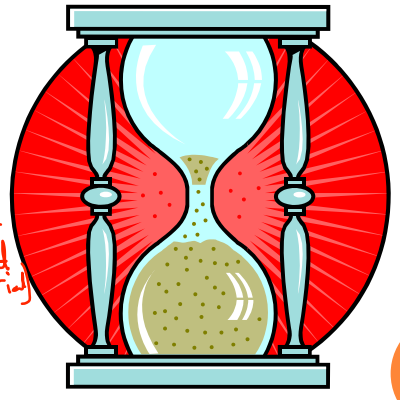
Selecting an Analytical Method

Introduction

- Types of Analyses:
  1. Quantitative → Conc. / percentage
  2. Qualitative → structure. → MWt → functional group



- Classification of **Analytical** Methods (Historical):
  1. **Classical** → titration → Find Conc. acid or base. → indicatoiur → indicator (coloured material)
  2. **Instrumental** → - Colour - taste. - smell. -



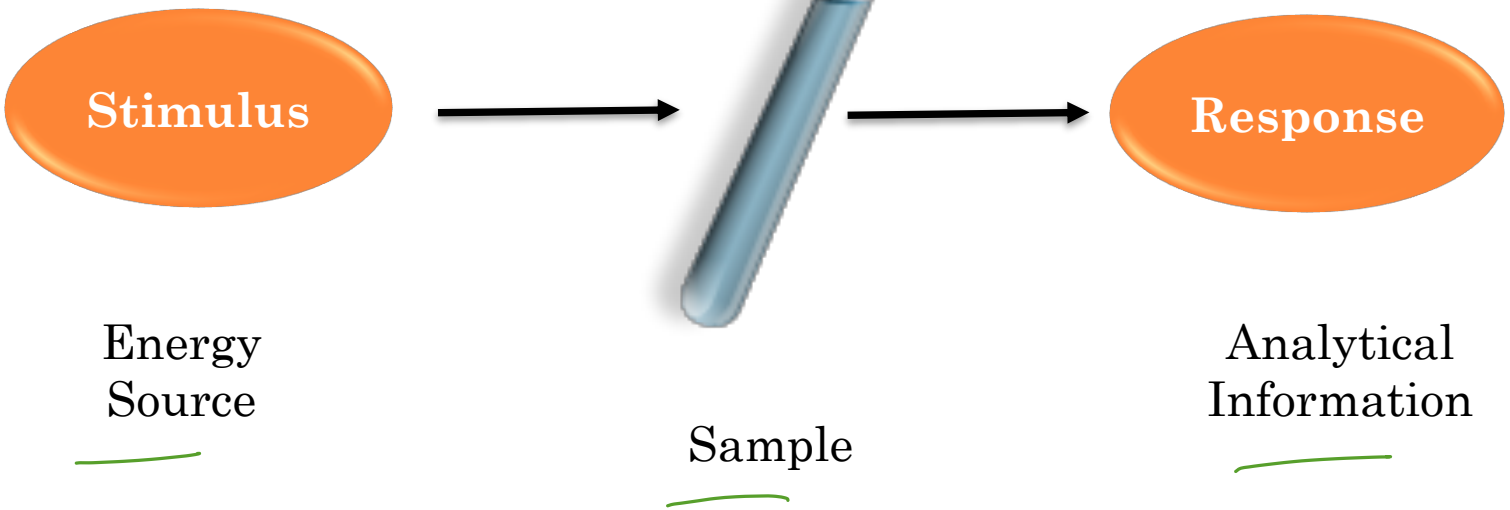
Classification of Analytical Methods

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.

Instruments For Analysis

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.



Types of Instrumental Methods

- According to characteristic properties employed to generate analytical signal:

1. Properties involving Electromagnetic Radiation:
   - Absorption, emission, scattering…
   - e.g. UV, IR, NMR, fluorescence, phosphorescence

2. Electrical Properties:
   - Electrical potential, charge, current or resistance.

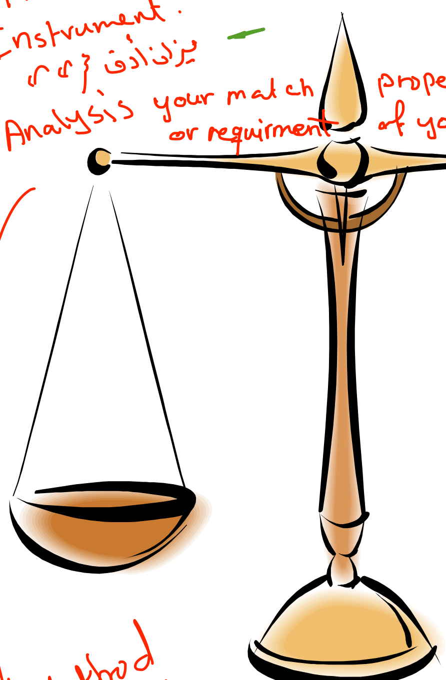
3. Miscellaneous:
   - Mass-to-charge ratio, mass, radioactivity.
   - e.g. Mass Spectrometry (MS)

Analytical Method Selection

How we select the method :-

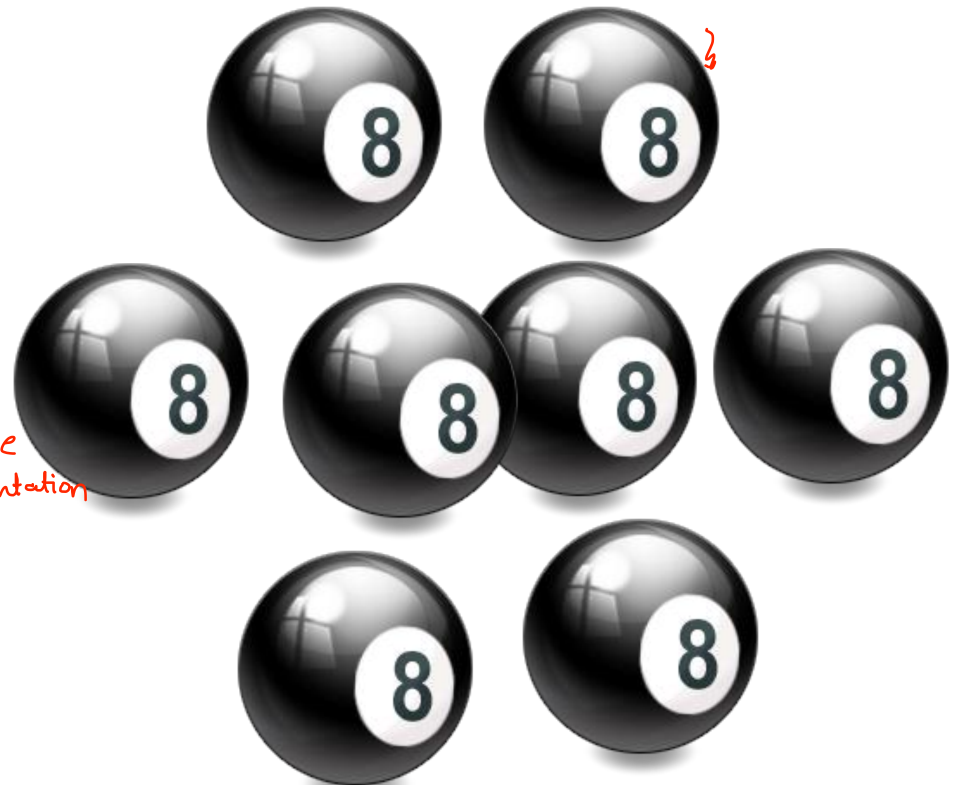
- characteris the problem
  - sample large or small
  - تركيز عالي او قليل
- the type of compound I can use to detect
- after charactrize your sample. goes → Instrument. بيزن ادق
- → Analysis your match properties of your sample or requirment of your analysis instrumentation

كلهم نفس الوزن وواحدة منهم وزنها اقل ب 19



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

6 characteristics

- 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 $= \frac{\text{absolute error}}{X_t} \times 100$
2. Amount / concentration of sample e.g. measure drug concentration in blood
3. Concentration Range — Instrument able to measure from lowest conc. to highest conc.
4. Interferences (e.g. Melamine contamination) — signal (Ex:-)
5. Matrix physical and chemical properties — Environment — blood → water.
6. Number of samples (cost, time, automation)

Sample (First part) — حتى نشخص العينة

Instrument (2nd part)

* Instrument.

Analytical Method Selection

- Instrument Performance Characteristics:
  1. Precision:
     - Agreement among data obtained in same way (replicate measurements)
     - 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?

Analytical Method Selection

- Precision can be expressed by calculating standard deviation

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

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

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

precision

- For method A in the previous example :

1. $\bar{x} = (70+80+90)/3 = 80$
2. $(x-\bar{x}) = 70-80,\ 80-80,\ 90-80 = -10,\ 0,\ 10$
3. $(x-\bar{x})^2 = 100,\ 0,\ 100$
4. $\Sigma(x-\bar{x})^2 = 100+0+100 = 200$
5. $s = \sqrt{\frac{200}{3-1}} = 10$

Method B

high precise
Low accuracy

high precise
high accuracy

Analytical Method Selection

- Instrument Performance Characteristics

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

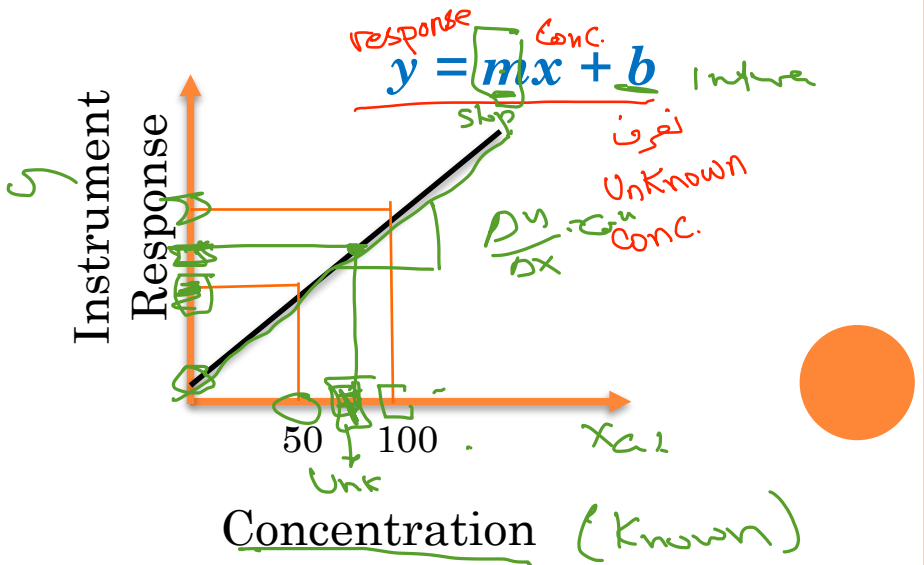
$$\text{bias} = \mu - x_t$$

Mean value — True value

Useful Terms

- **Blank** : is a sample that contains all of the components of the original sample except for the analyte.
- **Calibration curve:** is used by introducing several standards containing exactly known concentrations of the analyte into the instrument.

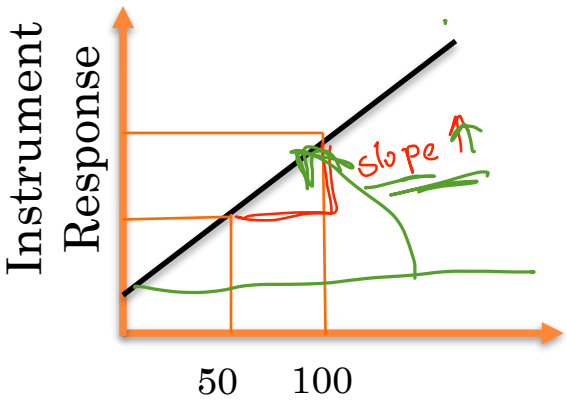
Concentration	Instrument Response
0	10
50	45
100	90
Unknow Conc	75

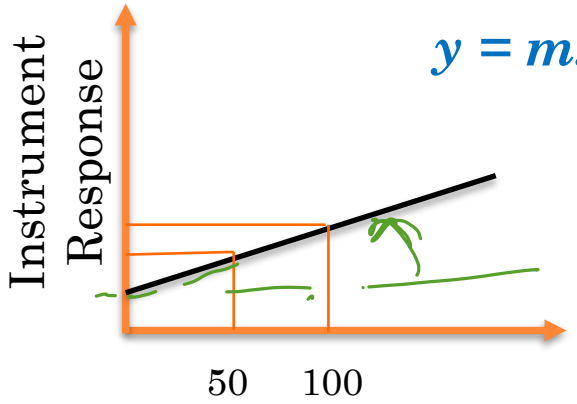


Analytical Method Selection

- Instrument Performance Characteristics
  - **Sensitivity**
    - Ability of method to distinguish between small differences in analyte concentration.
    - Depends on :
      - Slope of calibration curve
      - Precision (reproducibility)

Which method is more sensitive?





$y = mx + b$

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.

Detect is not quantified. by instrument

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 → 1ng/mL
LLOQ → 3 ng/mL

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

Standard deviation of the blank

Calibration curve slope

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

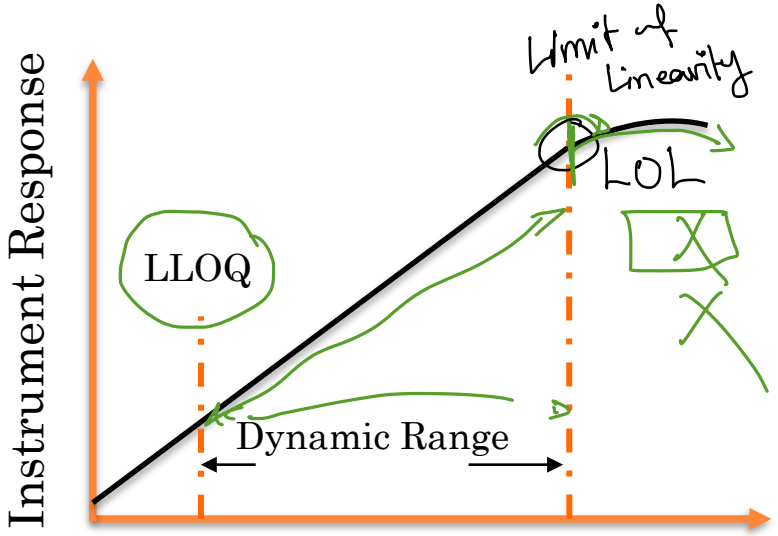
2ng/mL Alcohol /blood.
> DL Yes, there is this analyte

Sensitivity and DL? → 0.05 ng/mL < DL

Analytical Method Selection

- Instrument Performance Characteristics

5. **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.



Analytical Method Selection

- Instrument Performance Characteristics

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

Analytical Method Selection

- Instrument Performance Characteristics

  - Other characteristics for selecting analytical methods:

6. Speed → The time can be important if the result are required urgently time contributes to the cost.
7. Ease and convenience.
8. Skill required of operator.
9. Cost and availability of equipment.
10. Per-sample cost. [reagents, equipment / staffing cost]

* Safety → is so important parameter.
* Automation → reduce operator time, reproducibility

Notes

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

  - Calibration curves
  - Standard addition methods
  - Internal standard method

Analytical Process

- نعرف نوعها وماهي
- كيف نقوم بجمعها

Sampling

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

- مريض ← ناخذ دم
- عينات خضروات

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 لتخلص من matrix دون ان تتأثر العينة

Analysis

- Run standards and samples.

- Unknown
- blank
- standard Calibration

نحدد نوع الجهاز المناسب

Results

- Record and evaluate results of the analysis

تعريفه للشخص الذي طلب نتائج

Report and Evaluation

chapter 1 نهاية