INTERNATIONAL COLLEGE OF PHARMACEUTICAL INNOVATION

国际创新药学院

Tools in Analysis of Drugs and Medicines (I, II)

Course BSc (Pharm) or BSc (ATT)

Year 2024-2025 I

Module Medicines: Pharmaceutics 1 (MP1)

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LEARNING OUTCOMES

1. Summarise the tools used in the analysis of drugs and pharmaceutical dosage forms
2. Define accuracy and precision
3. Outline the role of spectroscopy in pharmaceutical science
4. Outline the role of chromatography in pharmaceutical science
5.

MEDICINES QUALITY

- Quality control is integral to all modern industrial processes

- Types of analysis
  - **Chemical**
  - **Physical**
  - **Microbiological**

- The quality of a medicine may deviate from the standard required, but one must be certain that the quality of the analysis itself is of a suitable standard.

STABILITY TESTING

DOSAGE FORM ATTRIBUTE TESTING

BIOANALYSIS

COMPATABILITY TESTING

APPLICATIONS OF ANALYSIS

QUALITY TESTING

DRUG ISOLATION

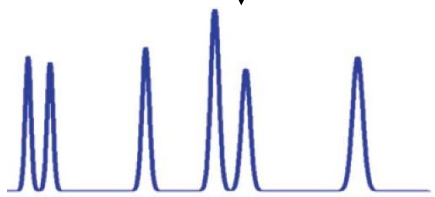
DRUG CHARACTERISATION

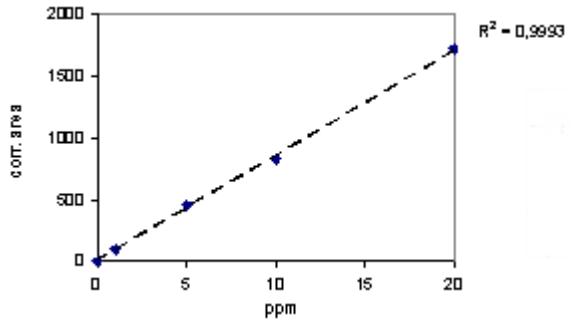
DRUG IDENTIFICATION

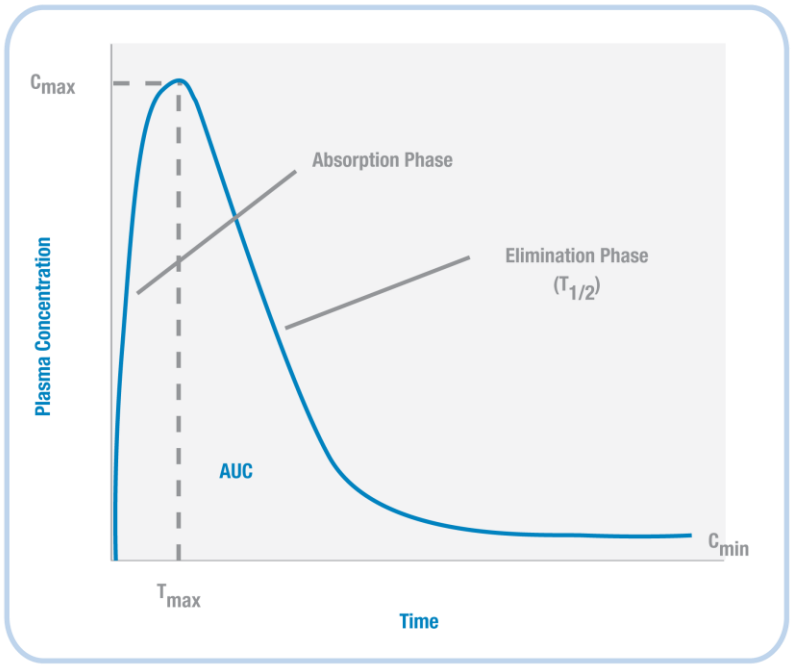
EXAMPLE OF SCIENTIFIC TOOLS IN R&D

- MICROSCOPY
- ELISA
- FLOW CYTOMETRY
- ELECTROPHYSIOLOGY
- BIOASSAY
- MOLECULAR DIAGNOSTICS
- BIOSPECIFIC INTERACTIONS
- PROTEOMICS
- BACTERIAL & MAMMALIAN CELL CULTURE
- PRE-CLINICAL ANIMAL MODELS

DRUG TESTING IN BLOOD







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PREFORMULATION

- Preformulation is the stage in drug and dosage form development before formal formulation.

Table 23.2 Macroscopic (bulk) properties and the techniques used to determine them

Derived property	Technique
Melting point	DSC or melting point apparatus
Enthalpy of fusion (and so ideal solubility)	DSC
Physical forms (polymorphs, pseudo-polymorphs or amorphous)	DSC, XRPD, microscopy
Particle shape Size distribution Morphology Rugosity Habit	Microscopy Particle sizing BET (surface area)
Density Bulk Tapped True	Tapping densitometer
Flow	Angle of repose
Compressibility	Carr's index Hausner ratio
Excipient compatibility	HPLC, DSC

Table 23.1 Molecular properties and the assays used to determine them

Property	Assay	Requirement of sample
Solubility* Aqueous Non-aqueous	UV	Chromophore
pK_a	UV Potentiometric titration	Acid or basic group
P_w^o / log P	UV TLC HPLC	Chromophore
Hygroscopicity	DVS TGA	No particular requirement
Stability Hydrolysis Photolysis Oxidation	HPLC, plus suitable storage conditions	No particular requirement

QUALITY TESTING

- Quality assurance (QA) and quality control (QC) departments develop and follow standard internal operating procedures directed toward assuring the quality, safety, purity, and effectiveness of drug products.

- **Specifications**: A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described. Specifications are one part of a total control strategy for the drug substance and drug product designed to ensure product quality and consistency.

- Example tests in quality management
  - Stability
  - Impurities
  - Residual solvent

DOSAGE FORM TESTING (EXAMPLES)

- **TABLETS**
- DISINTEGRATION TESTING (APPARATUS)
- FRIABILITY (APPARATUS)
- HARDNESS (APPARATUS)
- DISSOLUTION TESTING (APPARATUS)

- **CREAM**
- RHEOLOGY TESTING

SUMMARY OF ANALYSIS IN PHARMACEUTICAL SCIENCE

Box 1.1 Questions pharmaceutical analysis methods are used to answer

- Is the identity of the drug in the formulated product correct?
- What is the percentage of the stated content of a drug present in a formulation?
- Does this formulation contain solely the active ingredient or are additional impurities present?
- What is the stability of a drug in the formulation and hence the shelf-life of the product?
- At what rate is the drug released from its formulation so that it can be absorbed by the body?
- Do the identity and purity of a pure drug substance to be used in the preparation of a formulation meet specification?
- Do the identity and purity of excipients to be used in the preparation of a formulation meet specification?
- What are the concentrations of specified impurities in the pure drug substance?
- What is the concentration of the drug in a sample of tissue or biological fluid?
- What are the p*K*a value(s), partition coefficients, solubilities and stability of a drug substance under development?

TYPES OF ANALYSIS

- **QUALITATIVE ANALYSIS**: Analysis of a substance in order to ascertain the nature of its chemical constituents

- **QUANTITATIVE ANALYSIS**: Analysis of a substance to determine the amounts and proportions of its chemical constituents

VALIDATION OF ANALYTICAL PROCEDURES

- **ACCURACY**: Closeness of a measurement to the true value

- **PRECISION**: Closeness of two or more measurements to each other. The precision of an analytical procedures expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions
- **LEVELS OF PRECISION**
  - **REPEATABILITY**
  - **INTERMEDIATE PRECISION**
  - **REPRODUCIBILITY**

FACTORS THAT GIVE RISE TO INACCURACY AND IMPRECISION

Box 1.3 Some factors giving rise to imprecision and inaccuracy in an assay

- Incorrect weighing and transfer of analytes and standards
- Inefficient extraction of the analyte from a matrix, e.g. tablets
- Incorrect use of pipettes, burettes or volumetric flasks for volume measurement
- Measurement carried out using improperly calibrated instrumentation
- Failure to use an analytical blank
- Selection of assay conditions that cause degradation of the analyte
- Failure to allow for or to remove interference by excipients in the measurement of an analyte

ANALYSIS OF PARACETAMOL TABLETS: A CASE OF RANDOM VERSUS SYSTEMATIC ERROR

- Paracetamol tablets (500mg)

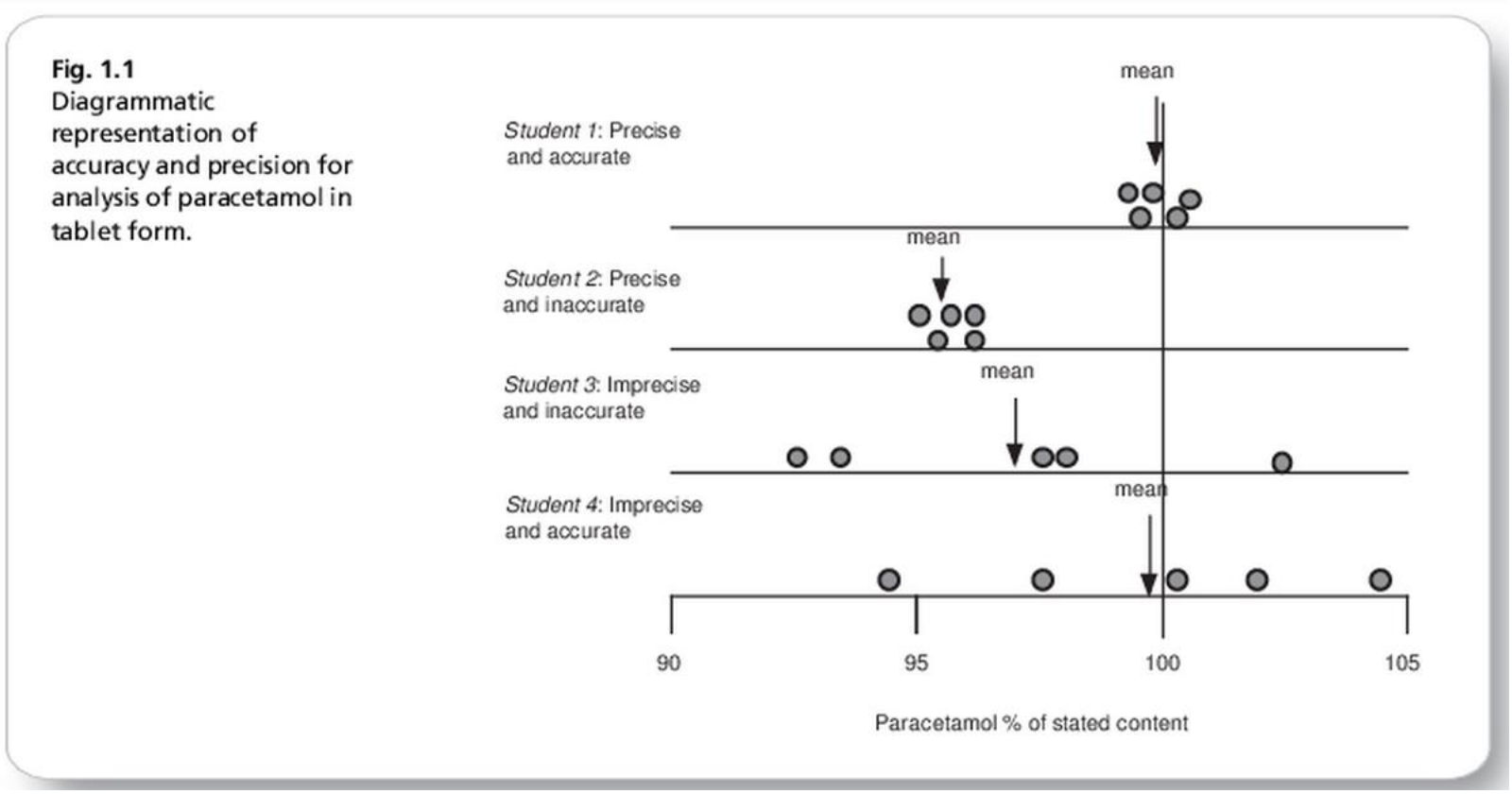

Fig. 1.1
Diagrammatic representation of accuracy and precision for analysis of paracetamol in tablet form.

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COMMON TERMINOLOGIES

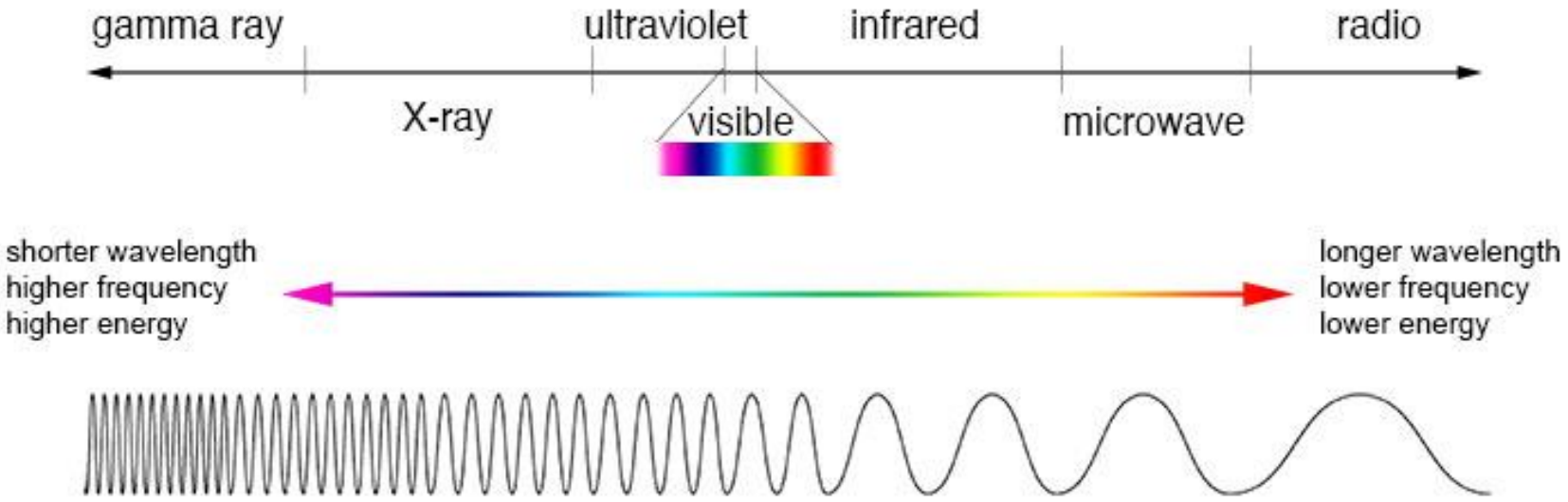
- ANALYTICAL BLANK
- CALIBRATION
- LIMIT OF DETECTION
- LIMIT OF QUANTIFICATION
- LINEARITY
- RANGE
- ROBUSTNESS
- SELECTIVITY
- SENSITIVITY
- WEIGHT BY DIFFERENCE

TITRIMETRIC AND CHEMICAL ANALYSIS METHODS

- **PRINCIPLE**: An analyte is chemically reacted with a standard soluton of reagent of precisely known concentration, the amount of which is used to estimate the purity of a sample.
- **Applications**
- Assay of drug, excipient and some formulations
- Specialist applications (e.g. Karl Fischer)
- **Adv**
- High precision, accuracy, robust, cheap, automatable, no calibration
- **Limitations**
- Non-selective, skilled operator
- Large amounts of sample and reagent

SPECTROSCOPY

- Spectroscopy is the study of the interaction between EMR and molecules
- EMR travels through space at defined frequency (hz) and wavelengths (m)

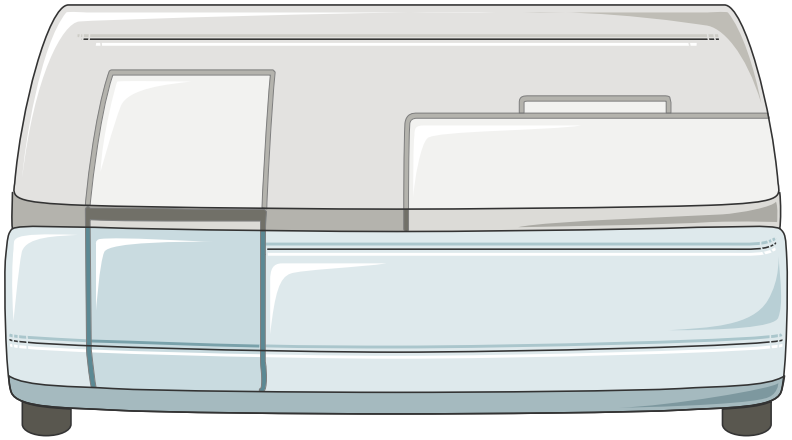


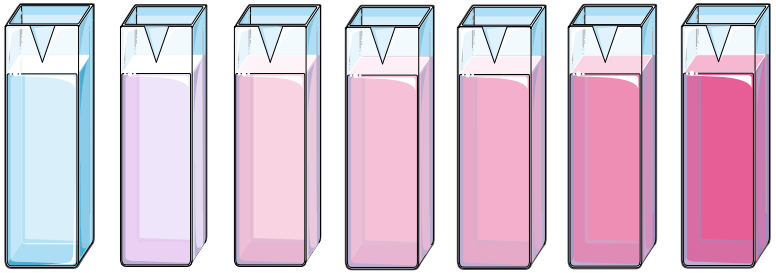
ULTRAVIOLET AND VISIBLE

- **PRINCIPLE**: Radiation in the wavelength of 200-700nm is passed through a solution of a compound. The electrons in the bonds become excited so that they occupy a higher quantum state and in the process absorb some of the energy passing through the solution. The more loosely held the electrons the longer the wavelength (lower the energy)
- **Application**
- Widely used method of quantification
- pKa, dissolution, solubility, release kinetics, reaction kinetics
- **Strengths**
- Easy to use, cheap, robust, precision
- **Limitations**
- Moderate selectivity
- Not applicable to all solutes

INSTRUMENTATION

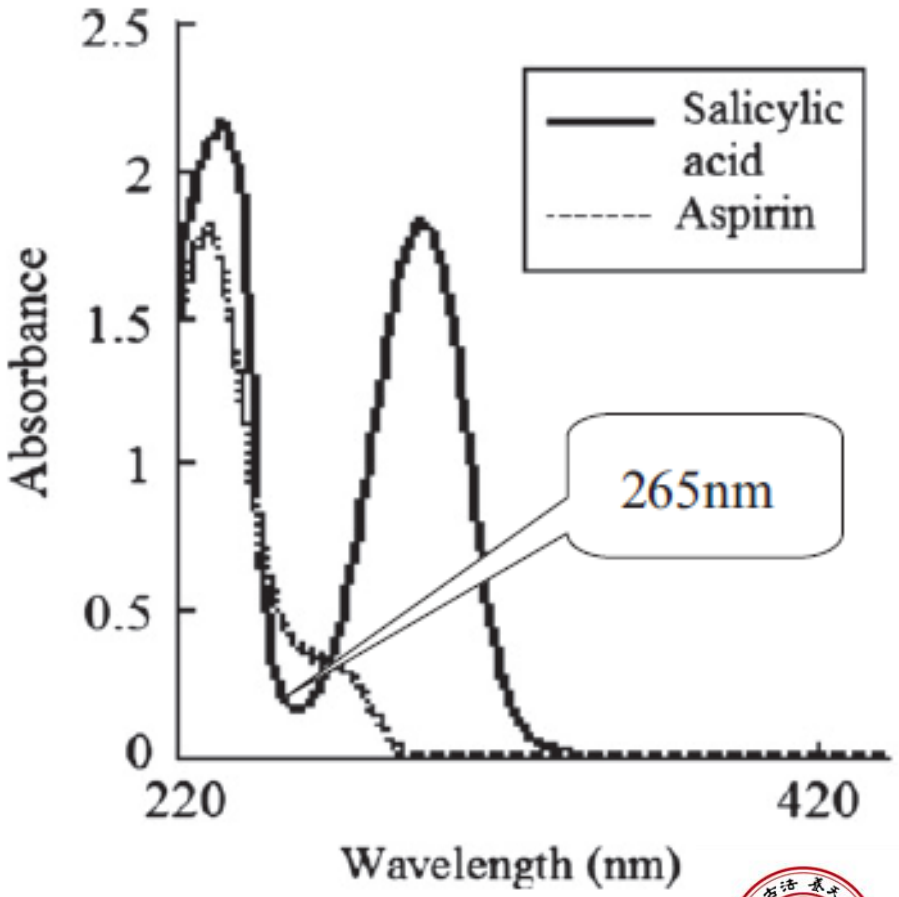
Absorption ∝ Concentration





CASE DRUG EXAMPLE: ASPIRIN

MONITORING THE HYDROLYSIS OF ASPIRIN TO SALICYLIC ACID



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IR SPECTROMETRY

DETAILED IN PCP1A: IR SPECTROSCOPY

- **PRINCIPLE**: EMR ranging between 400 cm^{-1} and 4000 cm^{-1} (2500 nm to 20000 nm) is passed through a sample and is absorbed by the bonds of the molecules in the sample causing them to stretch or bend. The wavelength of the radiation absorbed by the characteristics of the bond absorbing it
- **Application**
- Qualitative fingerprint check for excipients and drugs in manufacturing
- Preliminary compound identification
- Analysis in complex environments (creams, tablets etc)
- Detection of polymorphisms
- **Strengths**
- Complex fingerprint which is unique to the compound being examined
- **Limitations**
- Rarely used quantitatively due to sample preparation constraints
- Only useful for detection of gross impurities
- Technical knowledge required in sample preparation

OTHER SPECTROSCOPIC ANALYSIS

- ATOMIC ABSORPTION SPECTROSCOPY
- FLORESCENCE SPECTROSCOPY
- RAMAN SPECTROSCOPY
- NUCLEAR MAGNETIC RESONANCE

MASS SPECTROMETRY

- **PRINCIPLE**: Analyte is subjected to chemical ionisation or electron ionisation and resulting charged species are then accelerated into a vacuum chamber and separated on the basis of their mass to charge ratio (m/z ratio). The charged species produced in the spectrometer will generally include a range of different fragment ions, and under the right conditions, will also include the molecular ion, which is simply the intact test molecule minus 1 electron.
- Applications
- Drug identification and characterisation (molecular weight determination)
- Characterisation of impurities (in conjunction with GC and HPLC)
- Bioanalysis (in conjunction with GC and HPLC)
- Proteomics
- Limitations
- Expensive instruments, complexity that requires highly skilled operators,
- Less widely used in QC

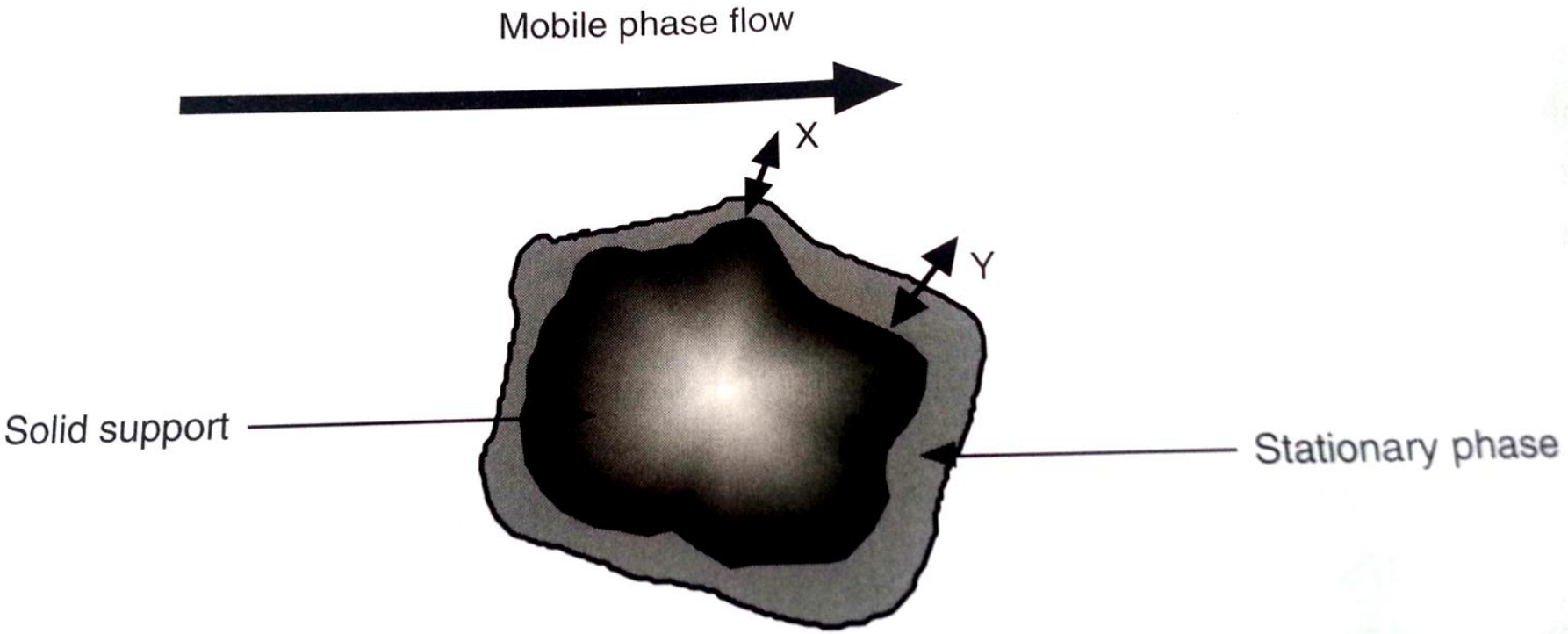
CHROMATOGRAPHY

- Chromatography is the most frequently use analytical technique in pharmaceutical analysis. Chromatography is the separation of the individual components of a mixture by exploitation of the difference in how analytes partition between a mobile phase and a stationary phase

- **TYPES**
- Column chromatography
- Thin layer chromatography
- Gas chromatography
- High pressure liquid chromatography
- RELATED TECHNIQUES: Capillary electrophoresis (not a type of chromatography)

- **MODES**
- **Analytical**: lower scale, analysis
- **Preparative**: higher scale, purification

SEPARATION THEORY



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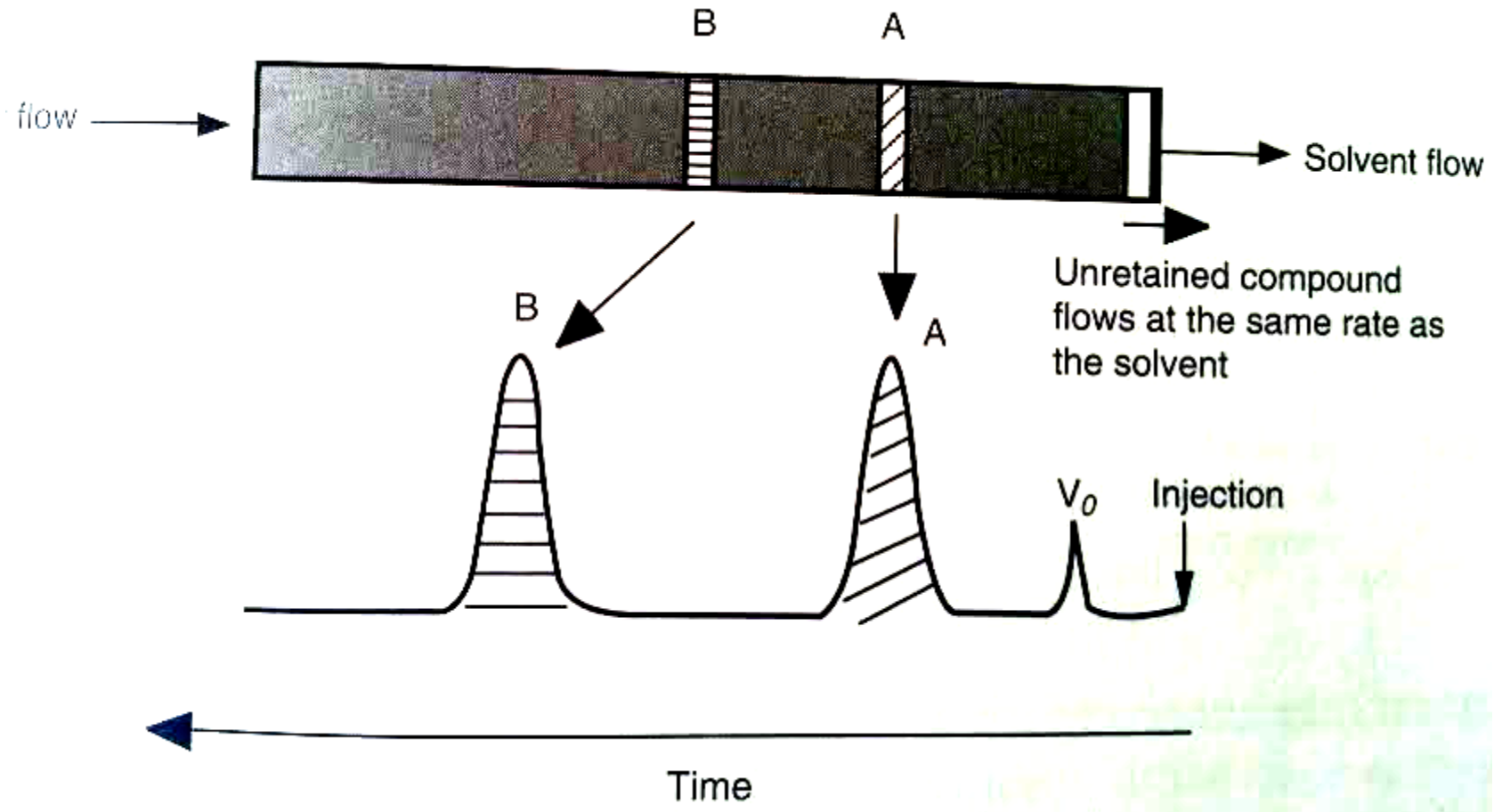
SEPARATION THEORY



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

- **PRINCIPLE**: A liquid mobile phase is pumped under pressure through a stainless steel column containing particles of stationary phase. The analyte is loaded onto the top of the column and separation occurs via the relative lengths of time spent by its components in the stationary phase. Monitoring exit from the column is recorder by a variety of detectors (e.g. an integrated UV VIS spectrophotometer)
- Applications
- Excellent tool for the quantification of drugs and excipients
- Monitoring stability
- Measurement of drug or their metabolites in blood
- Strengths
- Strong performance in analytical metrics (accuracy, precision, robustness)
- Large variety of separation and detection modalities
- Automatable
- Limitations
- Sample preparation remains a key requirement
- Large quantities of organic solvent waste (disposal costs)

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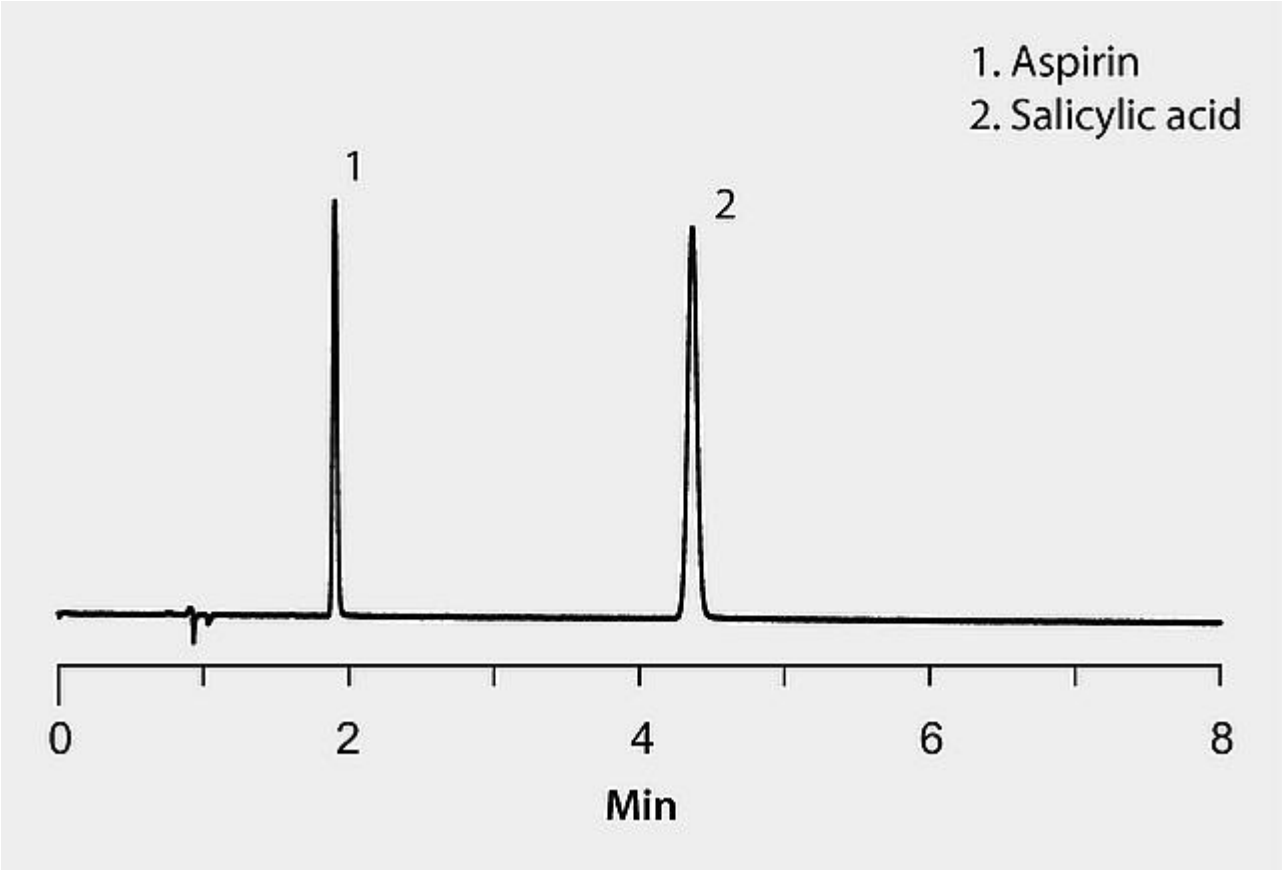
CASE DRUG EXAMPLE: ASPIRIN

RP-HPLC (UV detector) separation of aspirin from salicylic acid



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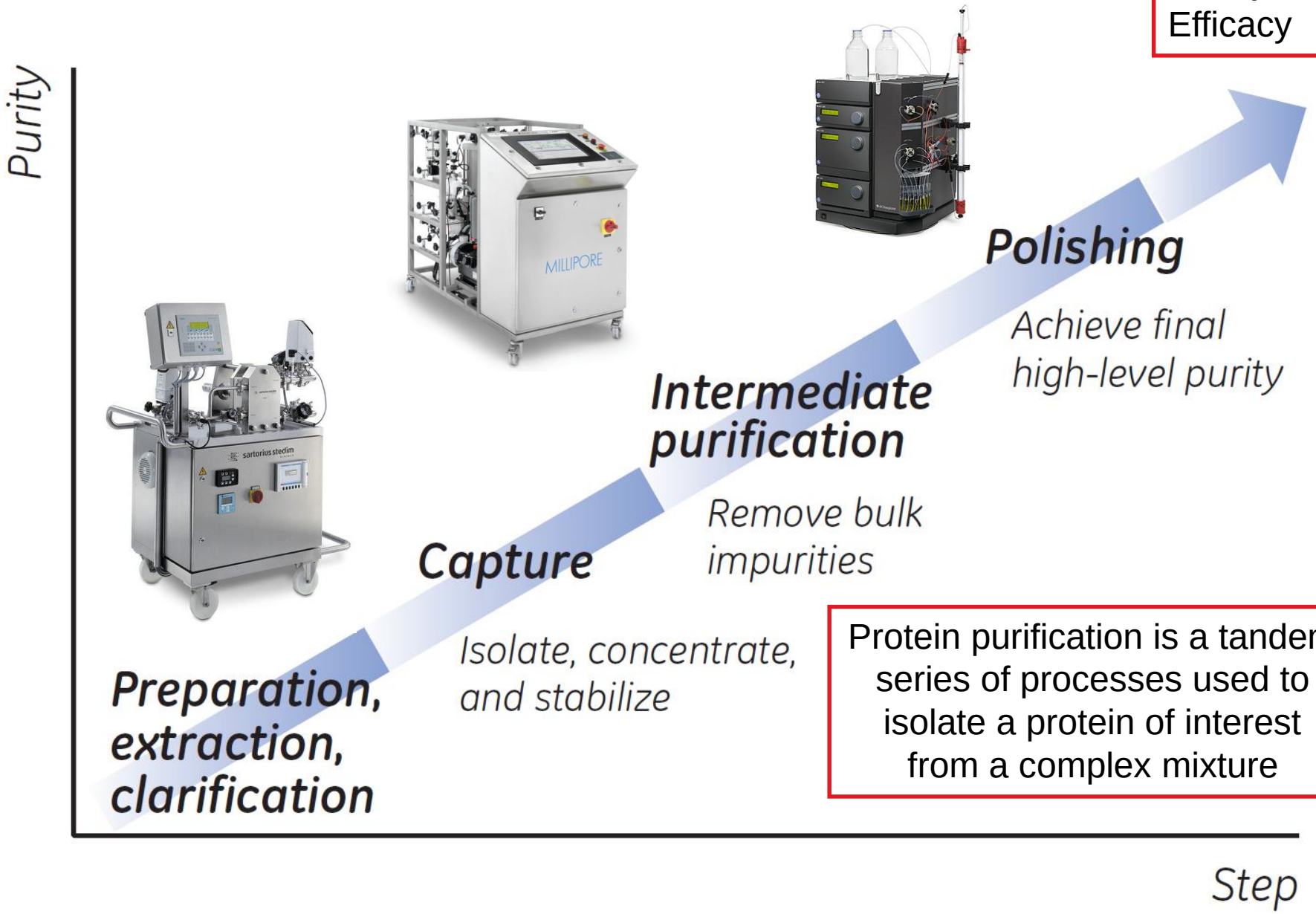
CHROMATOGRAPHY IN PURIFICATION

Quality
Safety
Efficacy



Protein purification is a tandem series of processes used to isolate a protein of interest from a complex mixture

OTHER TOOLS IN DRUG ANALYSIS

- GRAVIMETRIC ANALYSIS: Is a quantitative analytical technique that is based on the isolation of a substance by precipitation and weighing of the precipitate

- ELECTROANALYTICAL TECHNIQUES: Quantitative analysis by measurement of potential (Volts) and/or current (Amperes) in an electrochemical cells containing the analyte

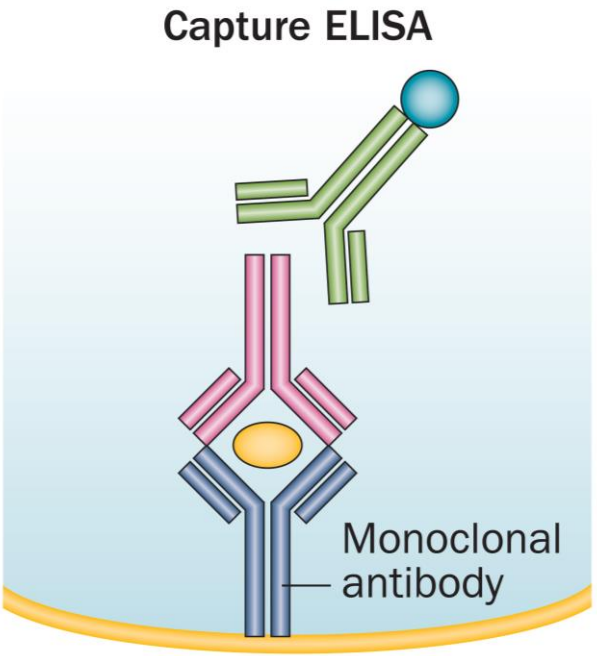
BIOANALYTICAL TECHNIQUES

- Bioanalytical science is the application of biological sciences to the study and analysis of biological samples and encompasses a multi billion pound global industry.

- **FORENSICS**
- **PHARMACEUTICAL R&D**
- **DRUG MONITORING**
- **FOOD SCIENCE**
- **MEDICAL DIAGNOSTICS**
- **CLINICAL LABORATORY TESTING**

IMMUNOASSAYS

- An **immunoassay** is a bioanalytical technique that uses the sensitivity and selectivity of the antibody-antigen reaction for the qualitative and quantitative analysis of analytes (drugs, endogenous biomolecules, metabolites)



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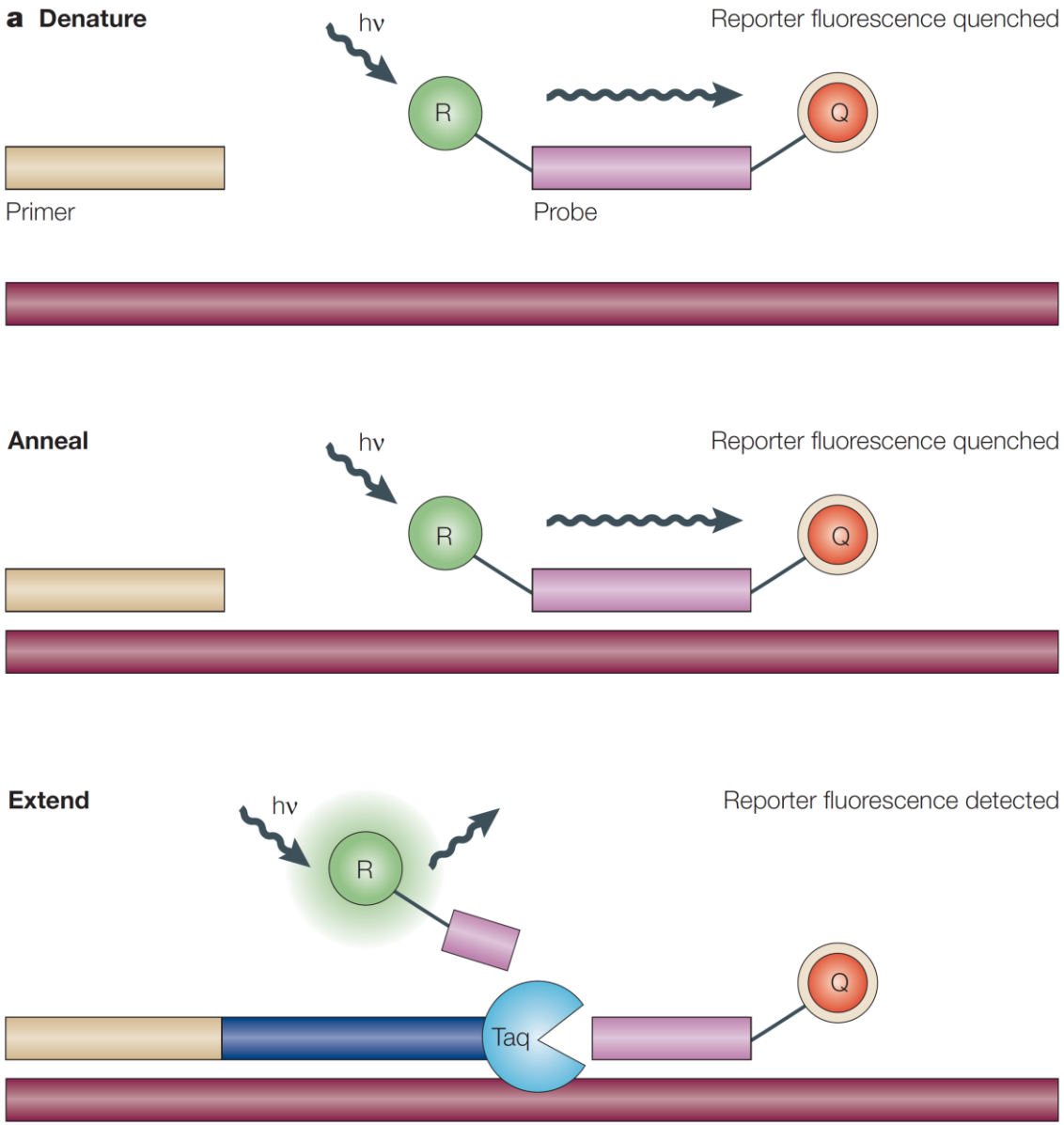
MOLECULAR DIAGNOSTICS



- E.G. TECHNOLOGY PLATFORMS FOR PHARMACOGENOMIC DIAGNOSTIC ASSAYS

BIOANALYSIS

- **Bioanalysis** is the quantitative measurement of drugs, their metabolites, biological molecules, and biotics in biological systems

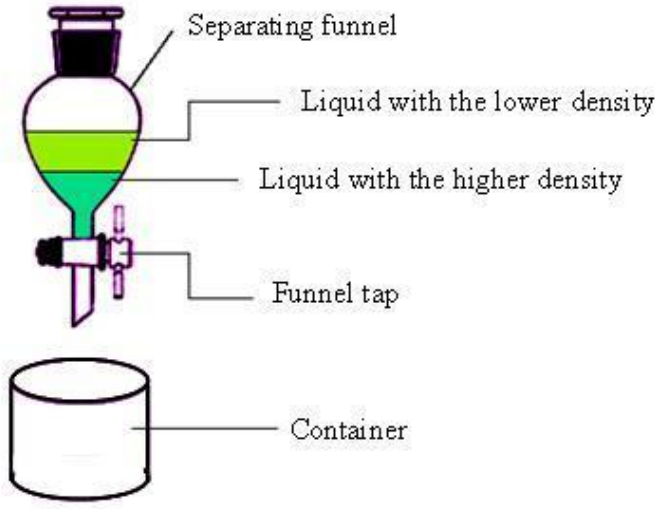
- **BIOLOGICAL SPECIMENS**
- BLOOD
- URINE
- SPUTUM
- FAECES
- SWEAT
- HAIR AND NAILS
- TISSUES
- EXHALED GASES

PRETREATMENT IN ANALYSIS

- Extraction is the removal of analyte from materials in the formulation matrix (tablets, creams) or from complex biological matrices (e.g. blood, sputum) which could (i) interfere in the analysis or (ii) potentially impede instrument function by non-adherence to operating specifications.

- The majority of samples must be pre-treated prior to analysis, and the methods used in extraction have significant bearing on the precision and accuracy of analysis.

- Common examples
- Solvent extraction
- Solid phase extraction



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