Drug analysis (small molecules)

Abdulghani ISMAIL
Chair Junior Professor
Institut Galien Paris-Saclay

abdul-ghani.ismail@universite-paris-saclay.fr

Rime Michael-Jubeli

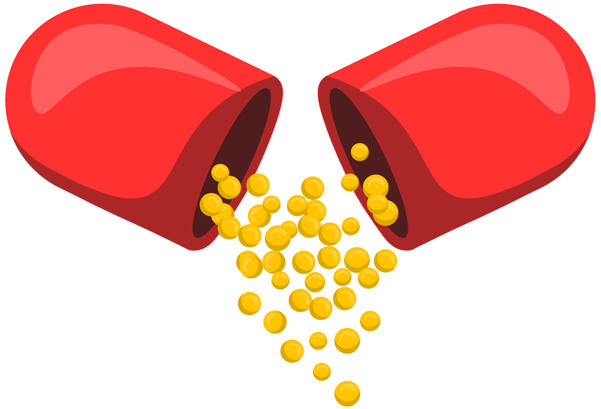
université PARIS-SACLAY | FACULTÉ DE PHARMACIE

CONTENT

Why is Pharmaceutical Drug Analysis Important?

Ensuring Safety and Efficacy

Analytical techniques help confirm that drugs contain the correct active ingredients, at the right dosage, without harmful impurities.



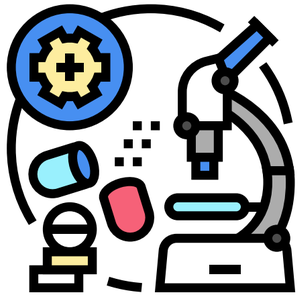
Quality Control:

Monitor consistency and quality during manufacturing, ensuring batch-to-batch reproducibility

EMA
EUROPEAN MEDICINES AGENCY
FDA APPROVED

Regulatory Compliance

Mandate rigorous analytical testing to approve new drugs and maintain public trust.

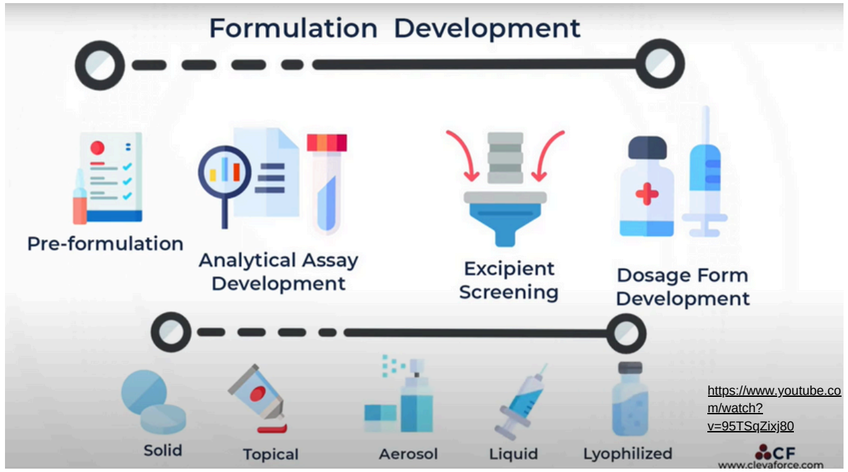


Drug discovery

Identification of new therapeutic compounds and their mechanisms of action.

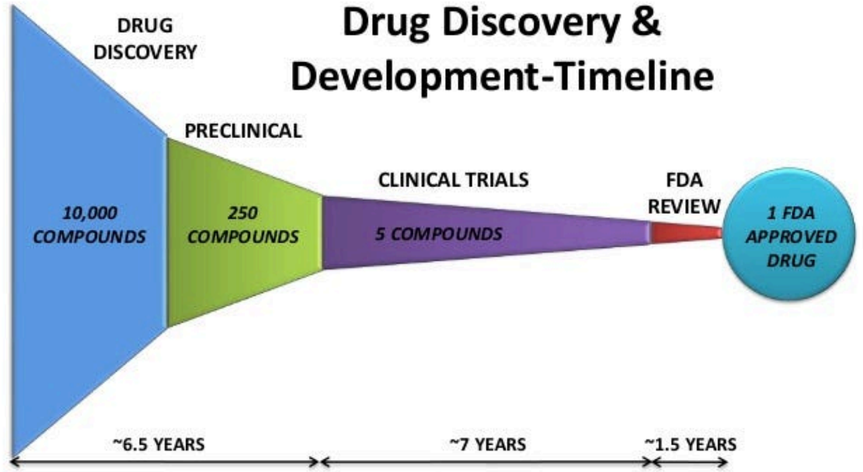
Applications in Real-World Contexts

FORMULATION DEVELOPMENT



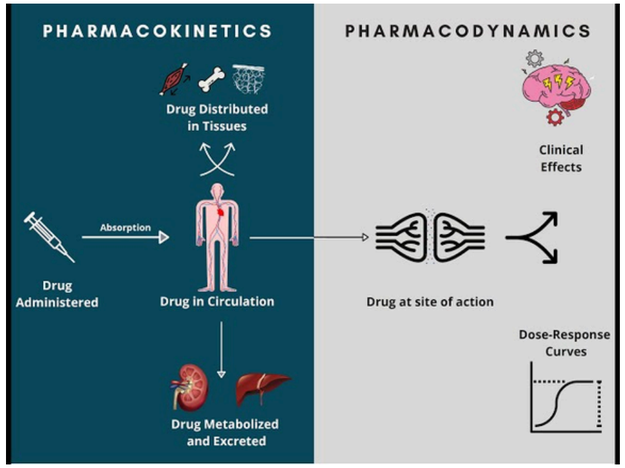
Ensuring stability and bioavailability of drugs.

DRUG DISCOVERY



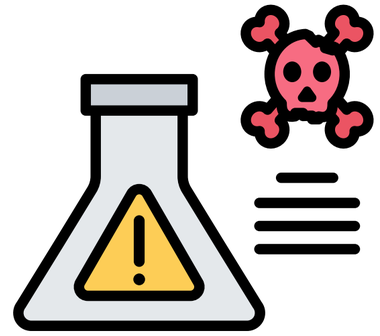
Identifying and characterizing potential therapeutic agents.

CLINICAL STUDY



Monitoring drug levels in biological fluids

TOXICOLOGY



Detecting and quantifying metabolites to assess safety

Drug discovery process

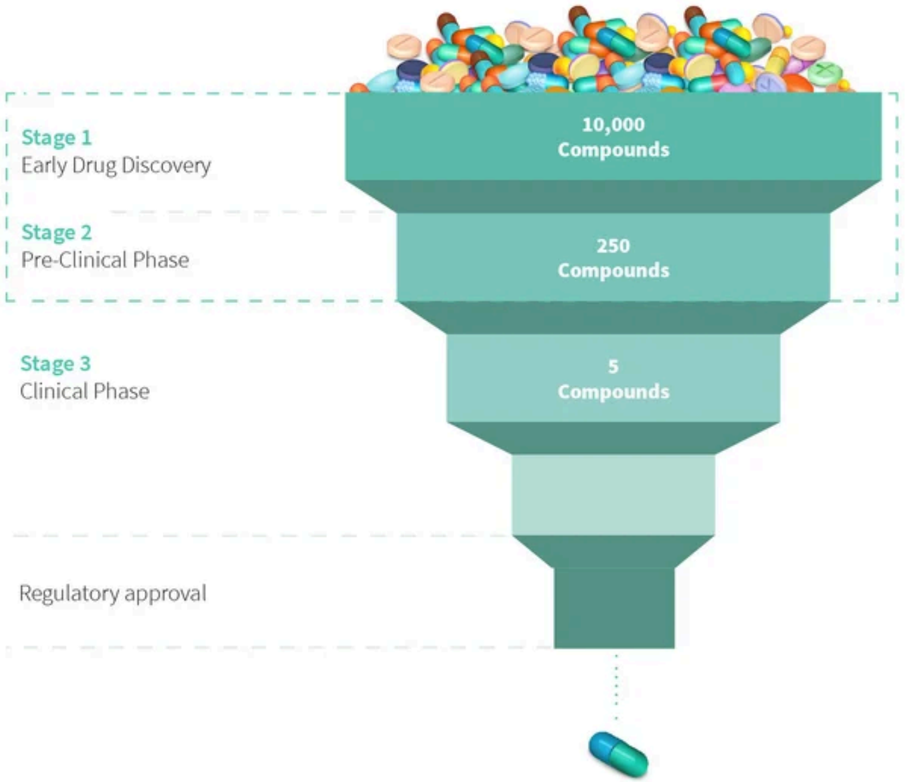
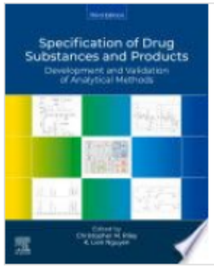


TABLE 1.3 Purpose of analytical methods by phase of development.

Clinical purpose	Pharmaceutical purpose	Purpose of methods
Early - To determine the safe dosing range and key pharmacological data (e.g., bioavailability and metabolism) in phase I trials involving a few healthy volunteers - To study efficacy in phase II trials in patients while continuing to test safety	Early - To deliver the correct bioavailable dose - To identify a stable, robust formulation for the manufacture of multiple, bioequivalent lots for phase II and III trials	Early - To ensure potency, to understand the impurity and degradation product profile, and to help understand key drug characteristics - To indicate stability and begin to measure the impact of key manufacturing parameters to help ensure DS or product consistency
Late - To prove efficacy, confirm safety, and obtain the desired label through phase III trials involving a large number of patients	Late - To optimize, scale-up, and transfer a robust and controlled manufacturing process for the commercial product	Late - To be robust, cost-effective, transferable, accurate, and precise for specification setting, stability assessment, and approval of final marketed products



Specification of Drug Substances and Products

Specification of Drug Substances and Drug Products is a fully comprehensive...

Google Books

The main objective is to verify the **identity**, **content**, **purity** and **stability** of drug substance, drug product and biomolecules using **qualitative and quantitative** analysis

Key techniques

Separative method

High-Performance Liquid Chromatography

Ultra-Performance Liquid Chromatography

Gas Chromatography

Thin-Layer Chromatography

High-Performance Thin-Layer Chromatography

Supercritical Fluid Chromatography

Capillary electrophoresis

Spectroscopic method

Mass Spectrometry

Ultraviolet spectroscopy

Infrared and NIR

Raman spectroscopy

Nuclear Magnetic Resonance

Inductively Coupled Plasma (ICP)

Atomic Absorption Spectroscopy (AAS)

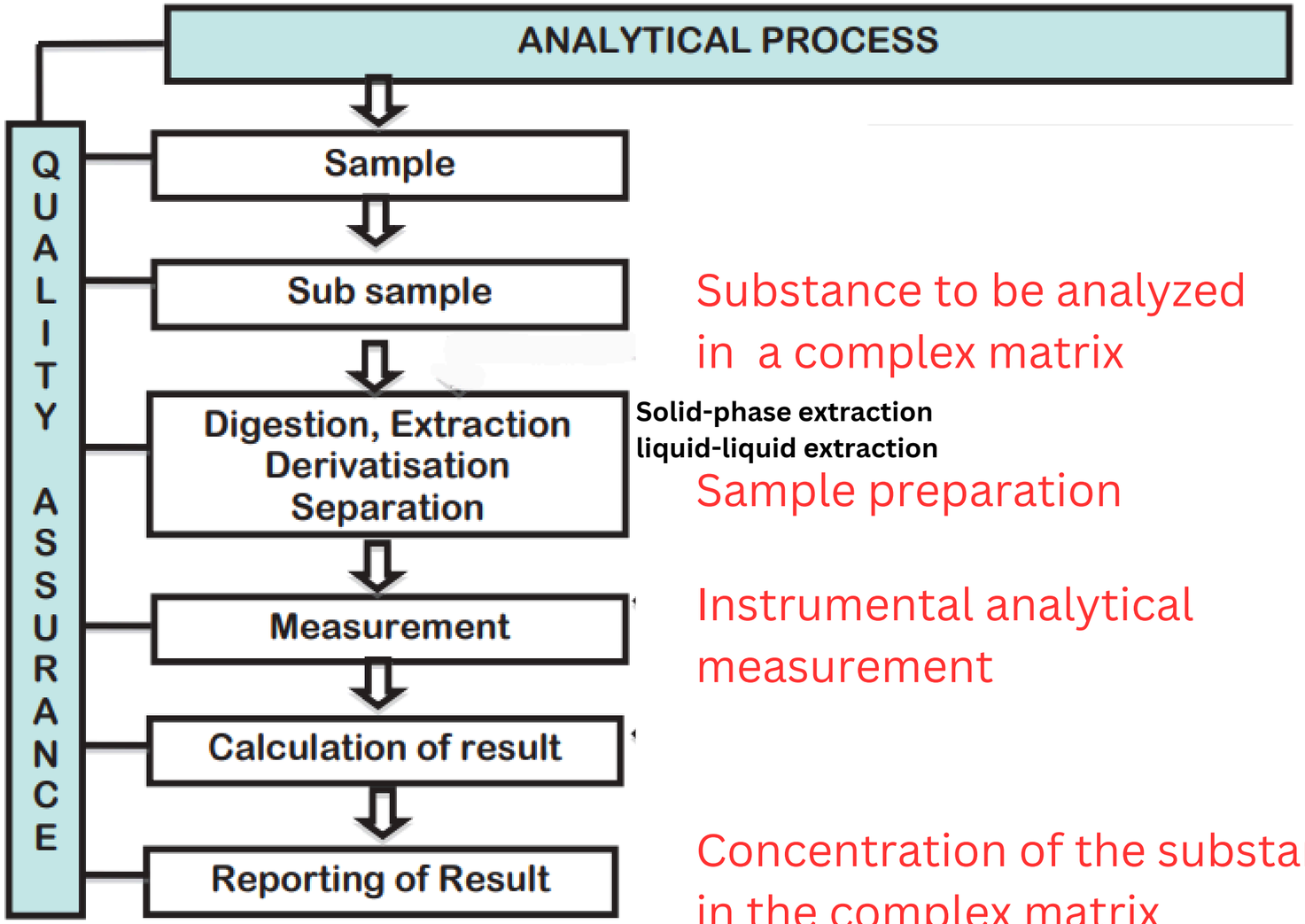
Hyphenated Techniques

LC-MS

GC-MS

ICP-MS

Workflow of drug analysis



Substance to be analyzed in a complex matrix

Solid-phase extraction
liquid-liquid extraction

Sample preparation

Instrumental analytical measurement

Concentration of the substance in the complex matrix

Zakaria, Osman, and Mohd Fazlin Rezali. "Reference materials as a crucial tools for validation and verification of the analytical process." Procedia-Social and Behavioral Sciences 121 (2014): 204-213.

Workflow of drug analysis

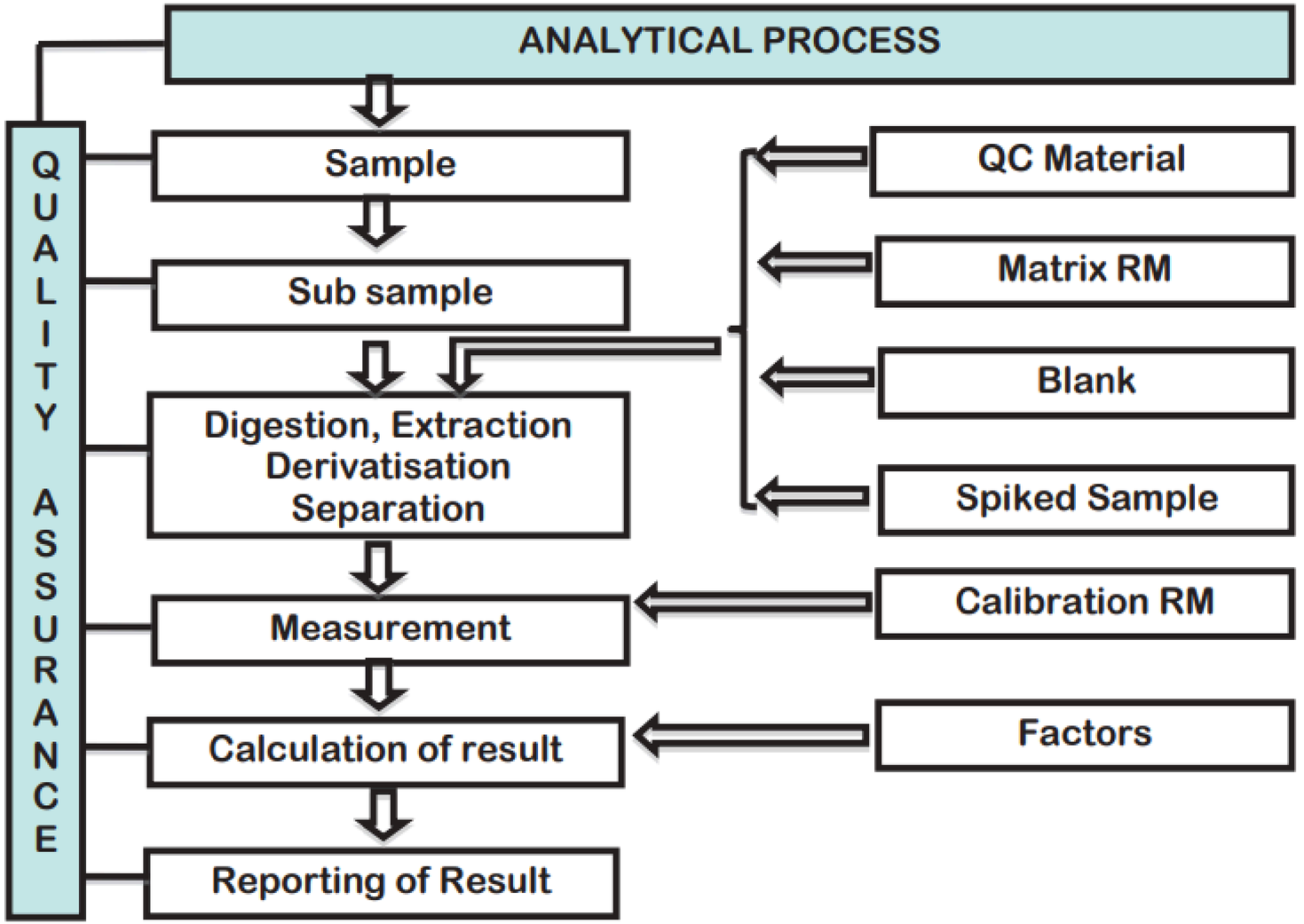
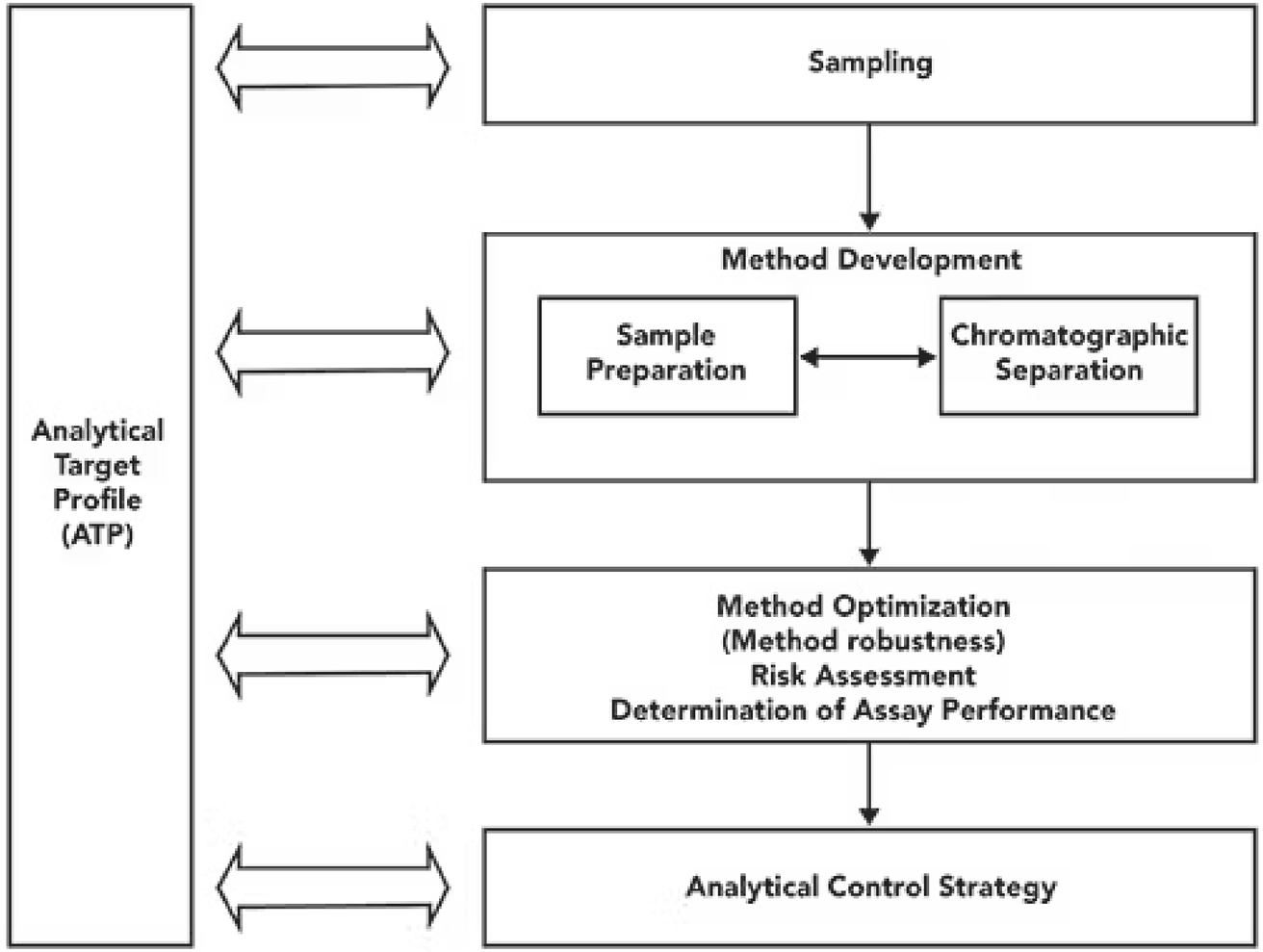


Fig. 1. Flow diagram of general steps of the total analytical process.

Zakaria, Osman, and Mohd Fazlin Rezali. "Reference materials as a crucial tools for validation and verification of the analytical process." Procedia-Social and Behavioral Sciences 121 (2014): 204-213.

Workflow of drug analysis

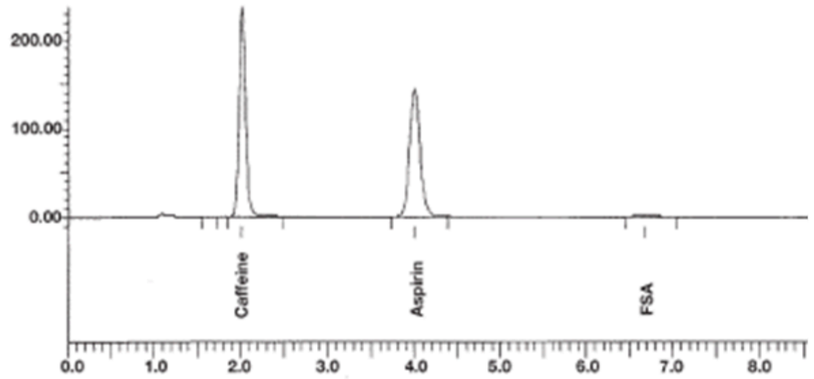


https://www.chromatographyonline.com/view/understanding-lifecycle-approach-analytical-procedures

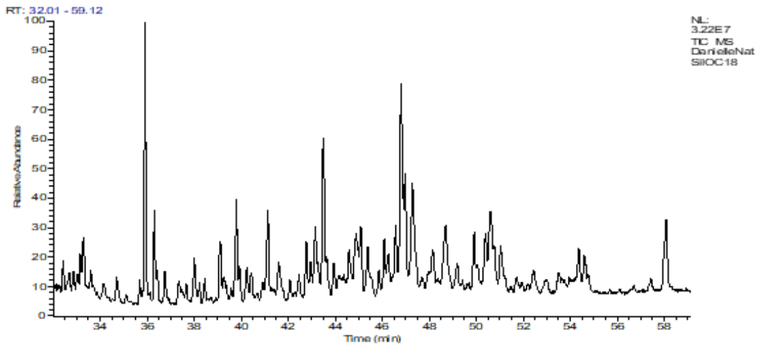
Analysis objectives

- Identification of compounds in a complex sample
  - Presence of pesticides in a biological fluid

- Limit test of a compound
  - Methylparaben concentration <0.1%
- Dosage of a compound
  - Glucose in the blood = 0.97g / L
  - AS in a pharmaceutical form
  - AS impurities
    - in the raw material?
    - in the end product?



- Profiling of a multi-compound raw material, a biological fluid, a food product…
  - Pharmacology, metabolism, toxicology
  - Phytochemical screening
  - Metabolomic analysis



Analytical constraints in Drug Analysis

Analyte’s Physicochemical properties

Solubility in liquid solvents

Polarity and electric charge

Size, shape and molecular volume

Presence of functional groups (ex: chromophores)

Volatility and thermal stability

Examples:

Determines solvent system, Aqueous or organic solvents

Impacts retention in chromatographic systems, Non-polar analytes favor reversed-phase HPLC

Detection system compatibility guides the choice of detector (UV for chromophores, MS for high sensitivity)

Restricts the use of GC for non-volatile compounds GC for volatile analytes

Analytical constraints in Drug Analysis

Analyte's Physicochemical properties

Solubility in liquid solvents

Polarity and electric charge

Size, shape and molecular volume

Presence of functional groups (ex: chromophores)

Volatility and thermal stability

Analytical objectives

Requirements for separative techniques (chromatography, electrophoresis....)

Choice of detection systems:

- UV, Fluorescence, MS
- Electrochemical deteciton

Solvent and buffer compatibility (UV-transparent, electro-spray ionisation-compatible, volatility...)

Analytical constraints in Drug Analysis

Analyte’s Physicochemical properties

Lipinski's Rule of Five: help researchers determine whether a molecule has the properties necessary to be a viable oral drug candidate

Four Key Rules:

- Molecular weight ≤ **500 Da** (Larger molecules may have difficulty crossing cell membranes)
- **Log P ≤ 5** (A value ≤5 indicates balanced hydrophilic and lipophilic properties which is important for membrane permeability)
- No more than **5 Hydrogen bond donors** (-OH and -NH) (Excessive hydrogen bonding reduces membrane permeability)
- No more than **10 (2*5) Hudrogen bond acceptors** (O and N atoms) (Too many acceptors can also hinder membrane crossing)

Exceptions:

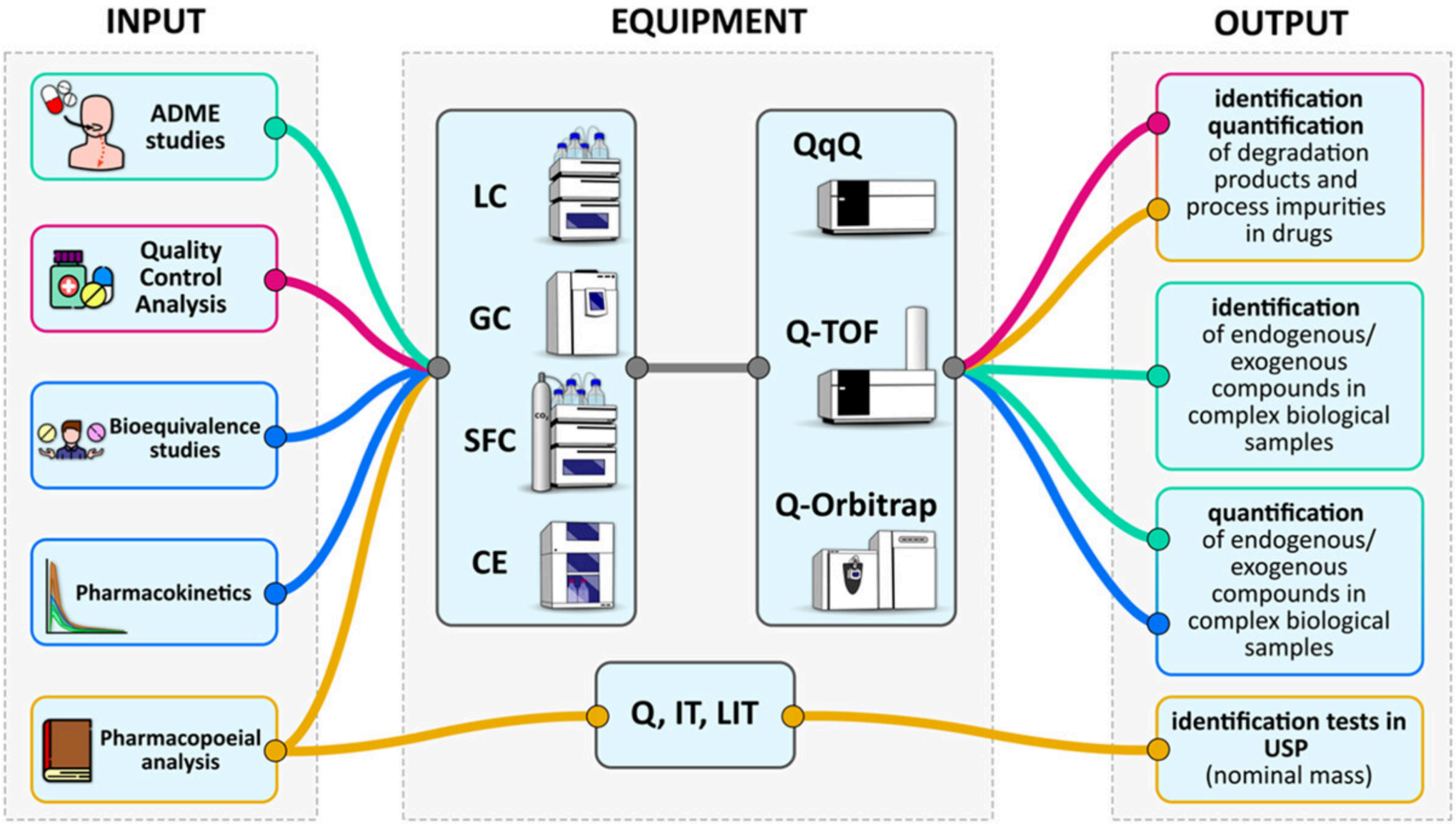
- Peptides and other large biomolecules
- Drugs that require specific transport mechanisms or are designed for non-oral delivery (ex: intravenous)

Analytical constraints in Drug Analysis

Most pharmaceutical substances are small molecules with moderate lipophilicity.

MS analysis for drug impurity identification and profiling

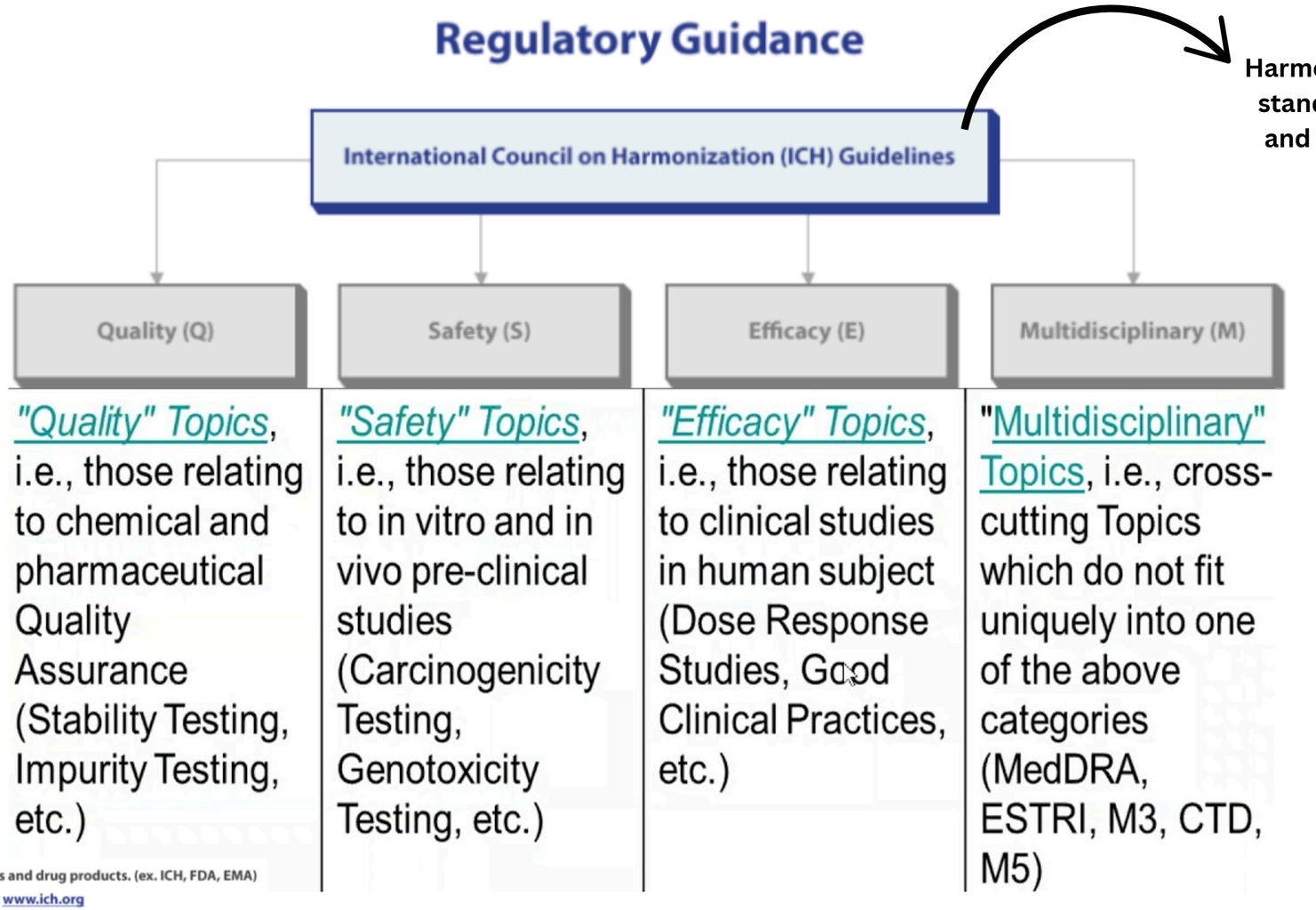
Sample
Small organics and amino acids
Mid-size organics
Large-size organics and peptides
Chiral organics
Salt determination
Residual Solvents



Khalikova, Maria, et al. Mass Spectrometry Reviews 43.3 (2024): 560-609.

Regulatory guidance for drug development

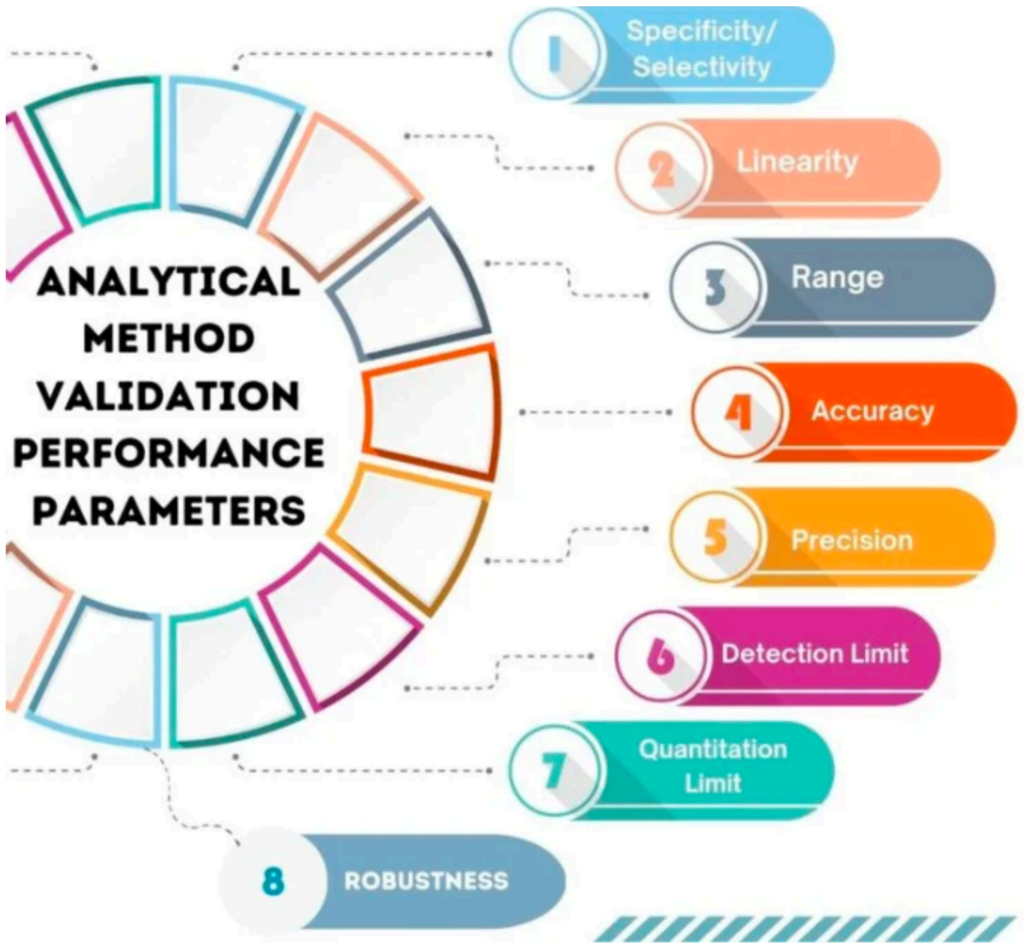
Drug development is complex and requires great care to ensure that the products we offer our patients are safe and effective. Consequently, drug development is highly regulated to ensure the appropriate controls are applied for all new chemical entities, whether they are marketed products or still in development.



Current regulatory guidelines apply to commercial drug substances and drug products. (ex. ICH, FDA, EMA)
ICH guidance database may be found at www.ich.org

Key Parameters in Analytical Method Validation

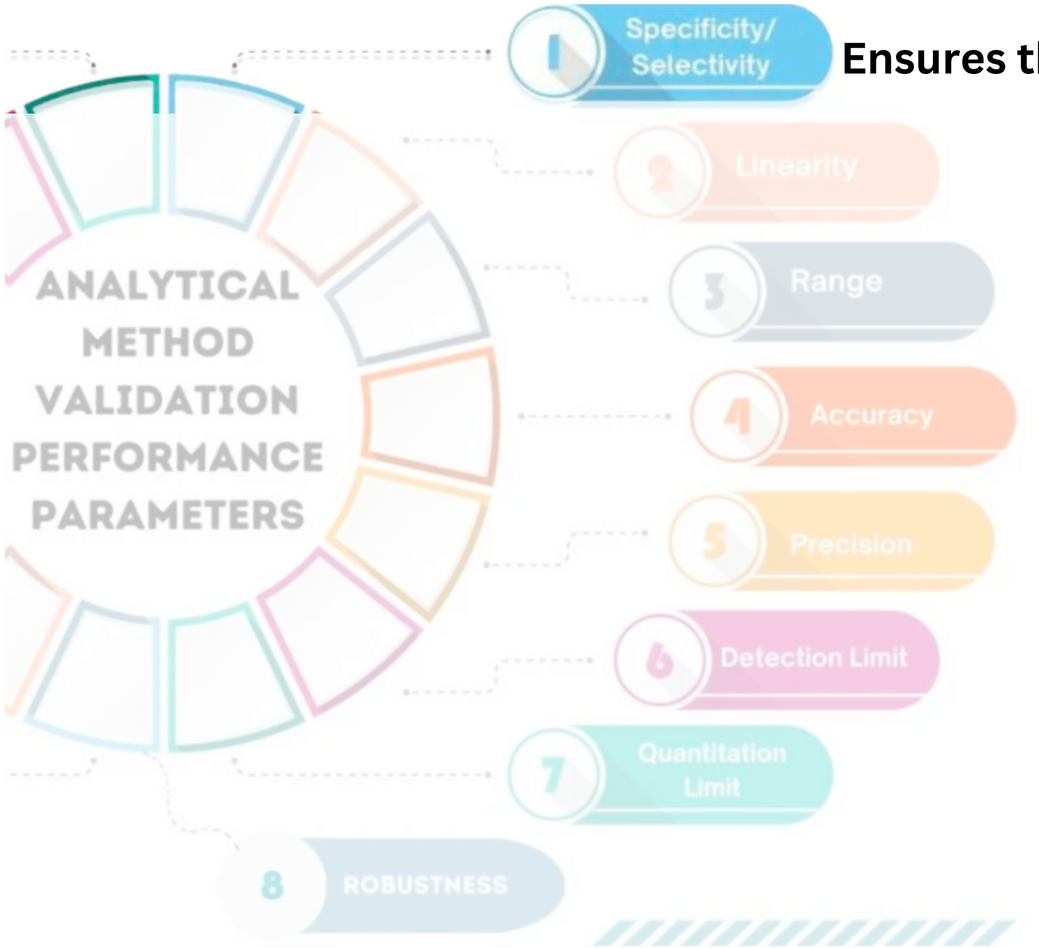
ICH



ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation

ICH



Ensures the method measures only the intended analyte without interference

Specificity is defined as the ability to assess the analyte **unequivocally** in the presence of components that might be expected to be present such as impurities, degradation products, or matrix components.

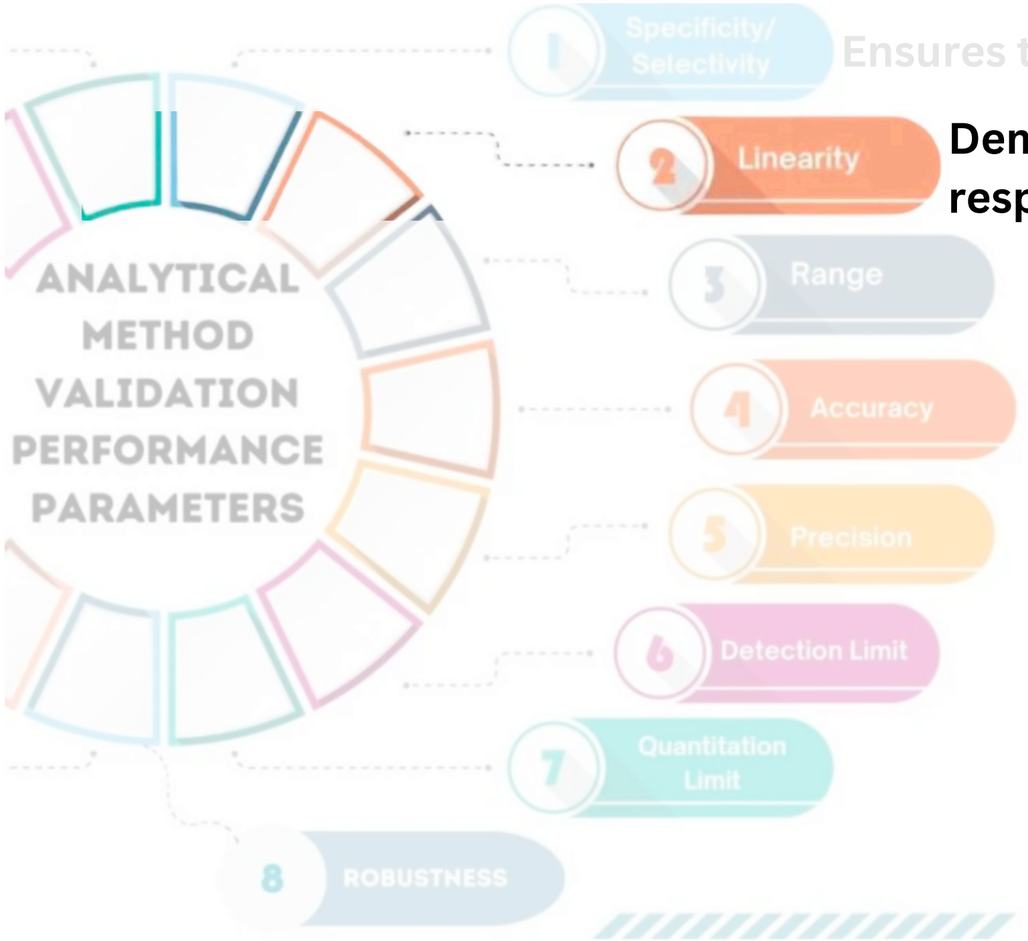
Selectivity is defined as the ability of an analytical method to differentiate and quantify the target analyte in the presence of other substances, including those with similar physical or chemical properties, such as structurally related compounds, isomers, or co-eluting species.

A selective method can differentiate the target analyte from **some interfering** substances, but it **might not exclude interference from all other components.**

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

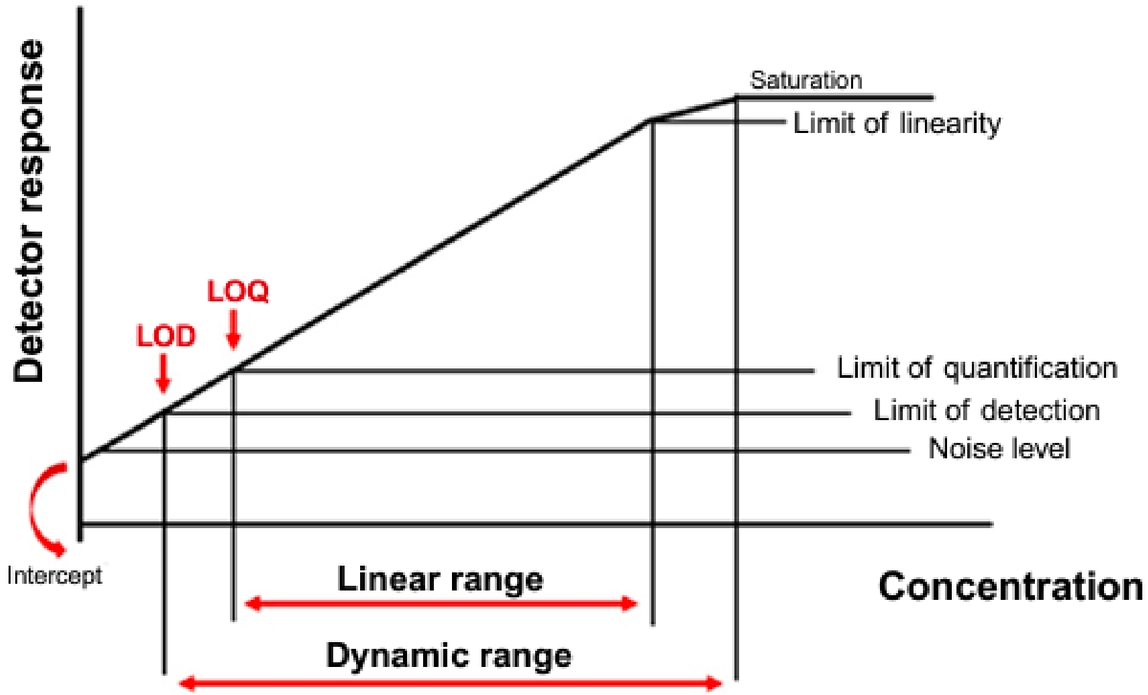
Key Parameters in Analytical Method Validation

ICH



Ensures the method measures only the intended analyte without interference

Demonstrates proportionality between the analyte concentration and response.

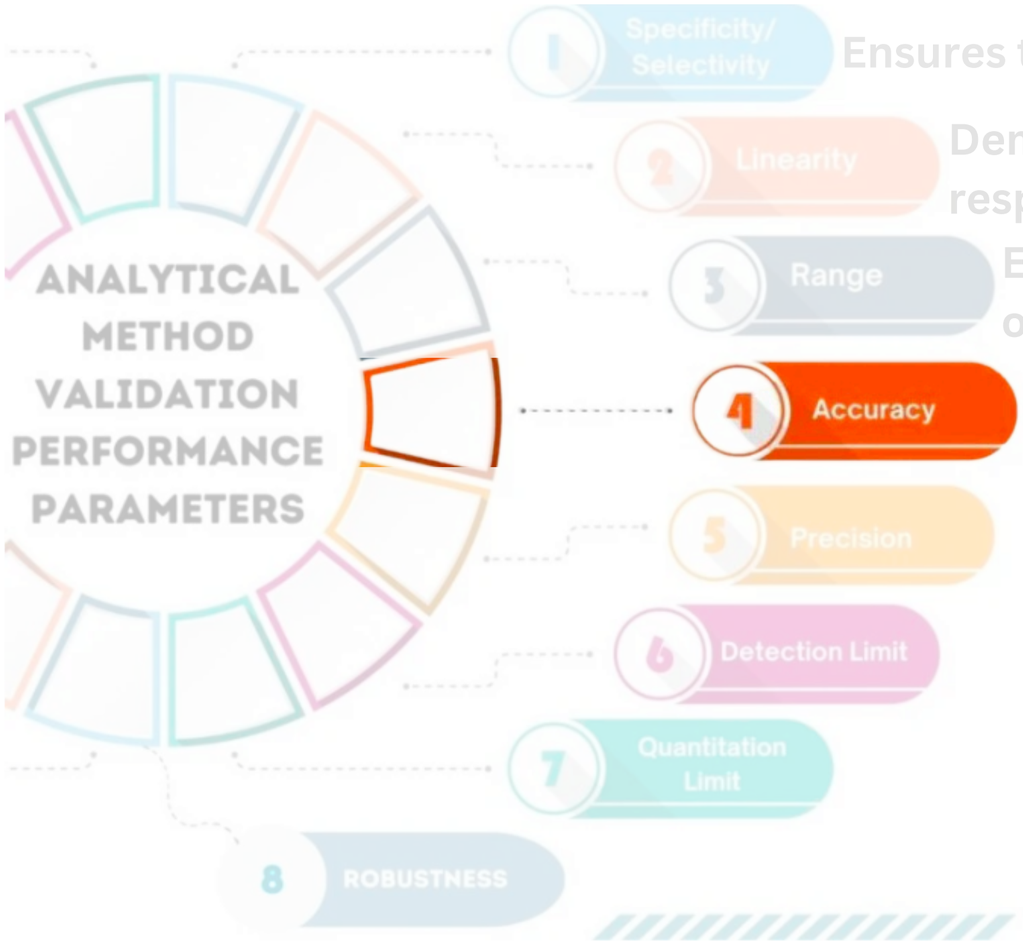


Kurbanoglu, Sevinc, Bengi Uslu, and Sibel A. Ozkan. "Validation of analytical methods for the assessment of hazards in food." Food Safety and Preservation. Academic Press, 2018. 59-90.

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation

ICH



Ensures the method measures only the intended analyte without interference

Demonstrates proportionality between the analyte concentration and response.

Establishes the practical boundaries where the method can reliably operate, considering not just linearity but also accuracy and precision.

Measures how close the result is to the true value

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation: Accuracy

- **Certified reference material (CRM):**

$$\%\,recovery = \frac{\bar{x}\ experimental\ measured}{certified\ value(CRM)} \times 100$$

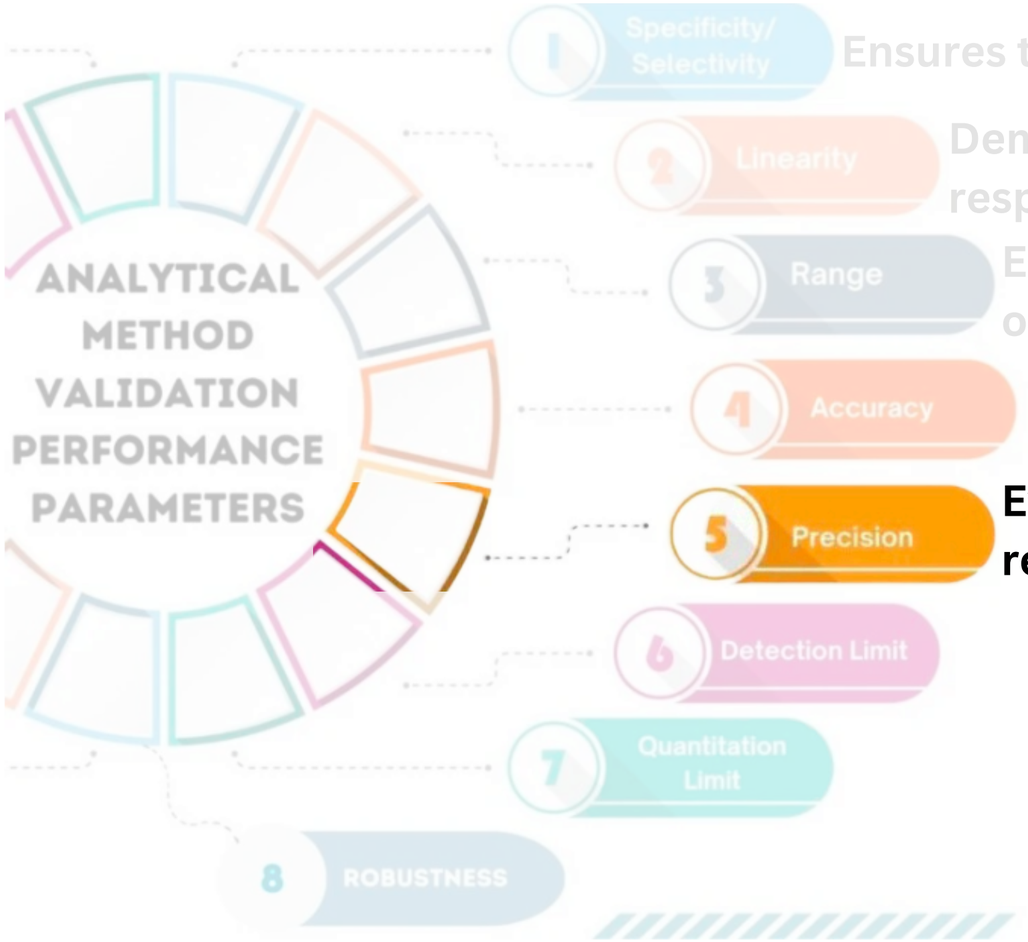
- **Recovery studies**

known quantity of substance added to the matrix (Placebo)

$$\%\,recovery = \frac{\bar{x}\ experimental\ measured}{theoretical\ amount\ spiked} \times 100$$

Key Parameters in Analytical Method Validation

ICH



Ensures the method measures only the intended analyte without interference

Demonstrates proportionality between the analyte concentration and response.

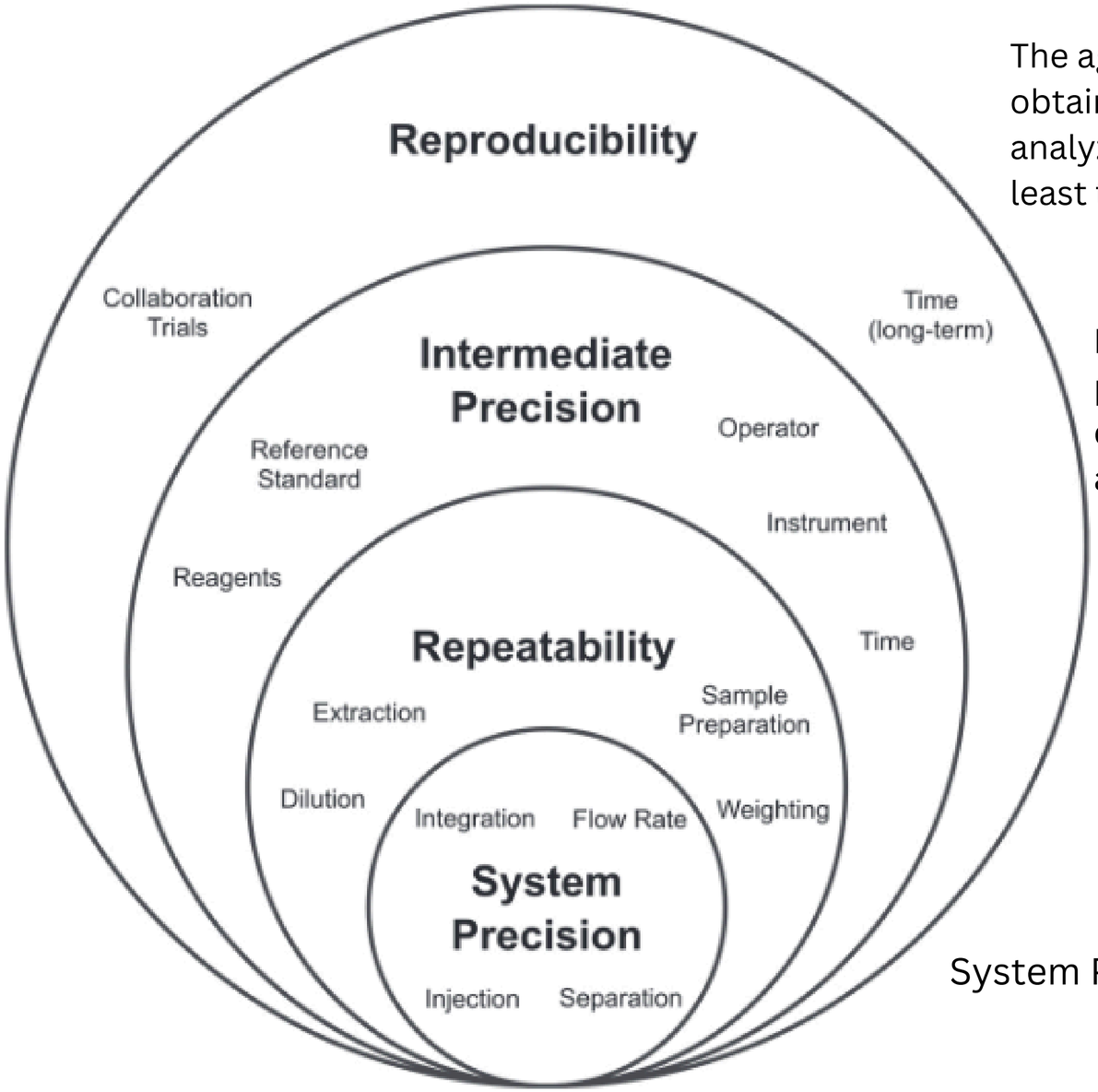
Establishes the practical boundaries where the method can reliably operate, considering not just linearity but also accuracy and precision.

Measures how close the result is to the true value

Evaluates the consistency of results (repeatability and reproducibility)

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation: Precision



The agreement among the results obtained in different laboratories that analyze homogeneous samples.(at least two laboratories)

Intermediate precision~inter-assay precision (analysis of similar samples on different days, with different analysts and different instruments)

Repeatability can be determined by performing a minimum of six replicates individually prepared at 100% of the test concentration, or nine determinations should be used with three different concentration levels (low, medium, and high) prepared in triplicate and covering the specified range for the procedure.

Repeatability ~ intra-day precision (evaluate the contribution of sample preparation)

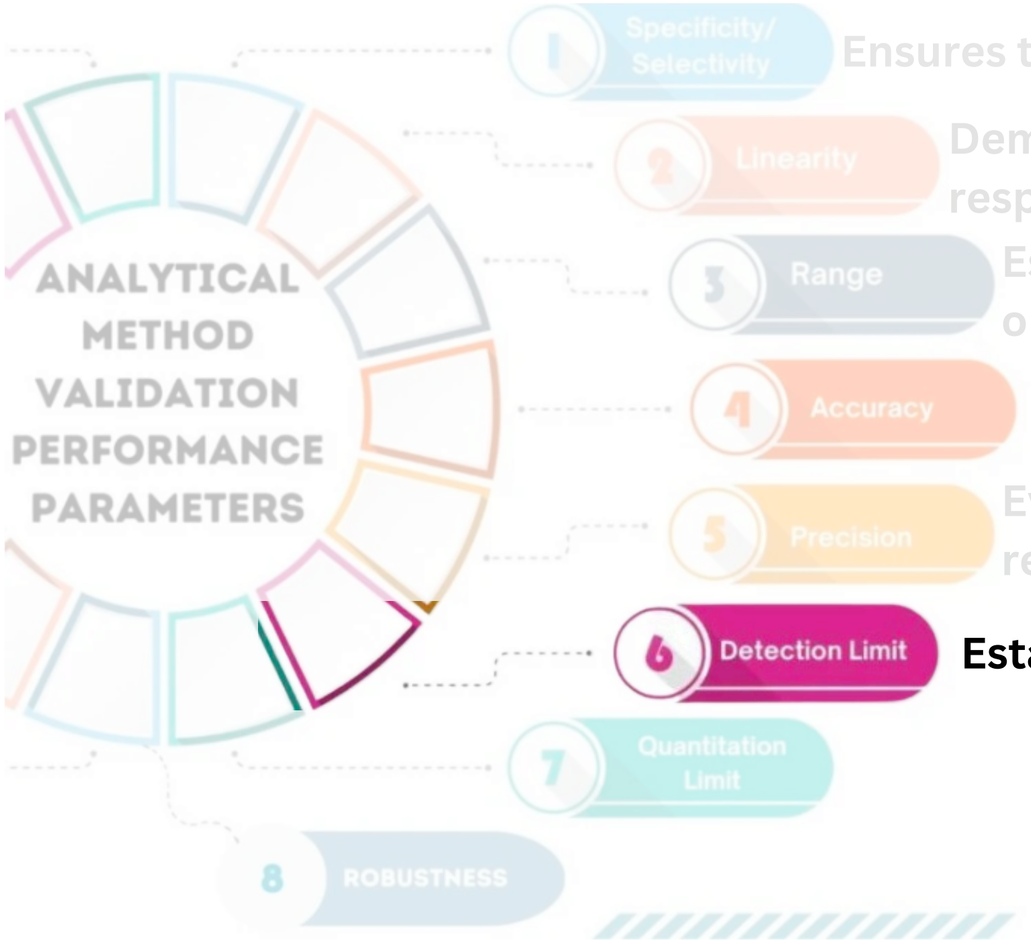
Acceptance limit commonly in pharma industry is upto 2%

System Precision ~ Instrumental precision

Marson, Breno M., et al. "Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods." Química Nova 43 (2020): 1190-1203.

Key Parameters in Analytical Method Validation

ICH



Ensures the method measures only the intended analyte without interference

Demonstrates proportionality between the analyte concentration and response.

Establishes the practical boundaries where the method can reliably operate, considering not just linearity but also accuracy and precision.

Measures how close the result is to the true value

Evaluates the consistency of results (repeatability and reproducibility)

Establish the smallest detectable amounts of the analyte.

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation: LOD

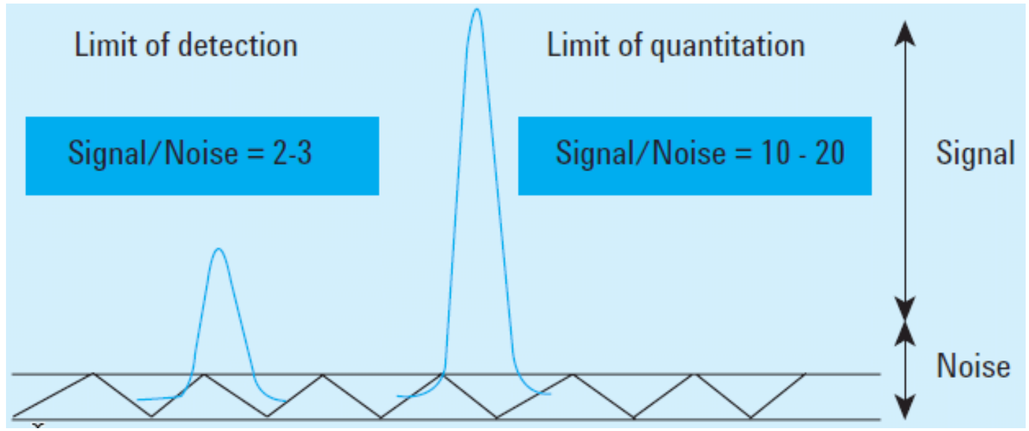
The LOD represents the lowest concentration of an analyte that can be reliably distinguished from the background noise.

Visual approach

- Analyze decreasing concentrations of the analyte.
- Determine the lowest concentration where the **analyte is visually distinguishable** from the blank or noise.

S/N approach

- Measure the signal (response) of a blank sample (noise).
- Measure the signal for a series of low-concentration standards of the analyte.
- The LOD is typically the analyte concentration that gives a signal at least 3 times the noise level (S/N ≥ 2-3).



Ravisankar, P., et al. "Validation characteristics and statistics in analytical method development." High technology letters 27.7 (2021): 76-88.

Key Parameters in Analytical Method Validation: LOD

Statistical approach

- Measure the signal and perform a calibration curve for a series of low-concentration standards of the analyte prepared in the complex matrix.
- The LOD is calculated for the regression curve

$$LoD = 3.3 \times SD / S$$

SD is the standard deviation of the intercepts of the calibration curve or the residual standard deviation of the curve

Slope of the calibration curve

Limit of Detection = 3.3σ / S

conc	signal
0	2.1
2	5.0
4	9.0
6	12.6
8	17.3
10	21.0
12	24.7

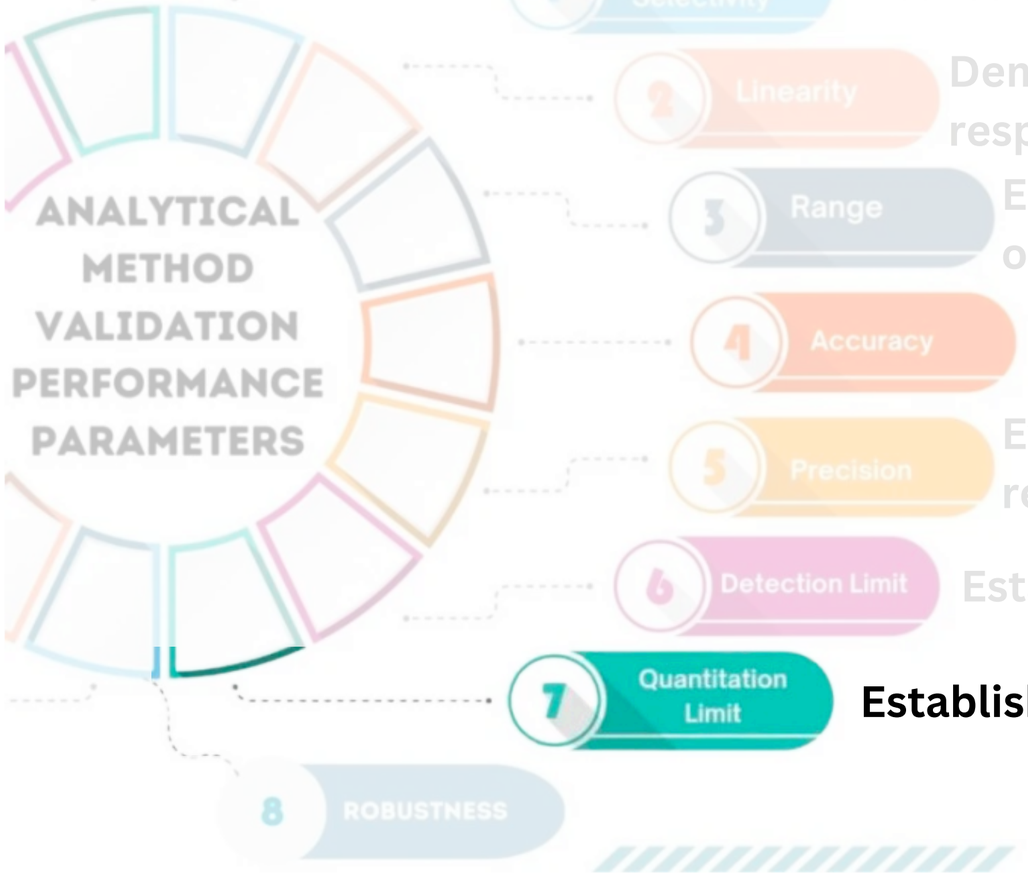
Excel

Regression Statistics	
Multiple R	0.9988
R Square	0.9977
Standard Error	0.4328
Observations	7
ANOVA	. . .
	Coefficients
Intercept	1.5178
conc	1.9303

(3.3 x 0.43) / 1.93 = 0.74
LOD = 0.74 ng/mL

Key Parameters in Analytical Method Validation

ICH



1 Specificity/ Selectivity — Ensures the method measures only the intended analyte without interference

2 Linearity — Demonstrates proportionality between the analyte concentration and response.

3 Range — Establishes the practical boundaries where the method can reliably operate, considering not just linearity but also accuracy and precision.

4 Accuracy — Measures how close the result is to the true value

5 Precision — Evaluates the consistency of results (repeatability and reproducibility)

6 Detection Limit — Establish the smallest detectable amounts of the analyte.

7 Quantitation Limit — **Establish the smallest Quantifiable amounts of the analyte.** **10x not 3.3x**

8 ROBUSTNESS

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation

ICH

1. **Specificity/ Selectivity** — Ensures the method measures only the intended analyte without interference
2. **Linearity** — Demonstrates proportionality between the analyte concentration and response.
3. **Range** — Establishes the practical boundaries where the method can reliably operate, considering not just linearity but also accuracy and precision.
4. **Accuracy** — Measures how close the result is to the true value
5. **Precision** — Evaluates the consistency of results (repeatability and reproducibility)
6. **Detection Limit** — Establish the smallest detectable amounts of the analyte.
7. **Quantitation Limit** — Establish the smallest Quantifiable amounts of the analyte.
8. **ROBUSTNESS** — Confirms the method's resilience under varying conditions.

ICH guideline Q2(R1) “Validation of Analytical procedures: Text and Methodology

Key Parameters in Analytical Method Validation: Robustness

The robustness of an analytical method describes its ability to withstand small and deliberate variations in analytical parameters, whilst maintaining acceptable precision and accuracy.The primary goal of robustness studies is to identify the method variables that are critical to ensure reliability and reproducibility of the results and to monitor routine analysis. Most experimental conditions are susceptible to normal fluctuations and occasional mistakes. The robustness provides essential information to predict the behavior of the results, maintaining the quality of the analysis, and occasionally guides troubleshooting during the daily execution of the method.

There is no standard that describes which parameters should be evaluated in the analysis of robustness. They must be determined by the analyst and will differ with different equipment and applied techniques. There are some suggestions of which parameters to choos

Separation technique	Factors
Liquid chromatography (LC)	Proportion of mobile phase constituents Mobile phase pH Buffer concentration Flow rate Column temperature Gradient elution - initial mobile phase Slope of gradient Stationary phase Column manufacturer Wavelength of detection
Gas chromatography (GC)	Type of column Injector temperature Column temperature Detector temperature Initial and final temperature Slope of the temperature gradient Carrier gas type/composition Gas flow rate Split or splitless conditions Split flow Liner type Column manufacturer Column stationary phase

Separation technique	Factors
Thin layer chromatography (TLC)	Eluent composition pH of the mobile phase Temperature Development distance Spot shape Spot size Batch of the plates Volume of sample Drying conditions (temperature, time)
Capillary electrophoresis (CE)	Electrolyte concentration Buffer pH Concentration of additives Temperature Applied voltage Sample injection time Sample concentration Rinse times Wavelength of detection

Sample preparation technique	Factors
Solid phase extraction (SPE)	Sorbent type Sorbent manufacturer Sorbent mass Sample mass or volume Wash solvent Elution solvent Evaporation temperature pH of sample pH of buffer constituents in solvents
Matrix solid phase dispersion (MSPD)	Sorbent type Sorbent manufacturer Sorbent mass pH of sample pH of buffer Sonication time Evaporation temperature Wash solvent Elution solvent Sample mass or volume

Adapted from Karageorgou, Heyden and Dejaegher [50-52]

Key Parameters in Analytical Method Validation

S. No	Parameter	Acceptance criteria
1	Accuracy	% Recovery 98 – 102 % %RSD of recovery concentrations must be < 2
2	Precision	RSD < 2%
3	Range	Concentration where data can be reliably detected(80 – 120%)
4	Specificity	No interference
5	Linearity	Correlation coefficient – NLT 0.999
6	Detection Limit	S/N > 2 or 3
7	Quantitation Limit	S/N > 10
8	Ruggedness	Should meet all system suitability parameters
9	Robustness	RSD < 2%

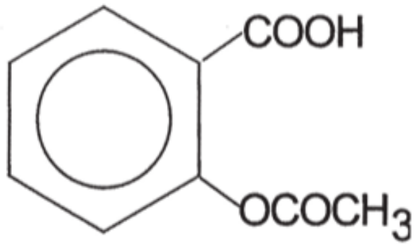
Acceptance criteria of validation parameters for HPLC

Ravisankar, P., et al. "Validation characteristics and statistics in analytical method development." High technology letters 27.7 (2021): 76-88.

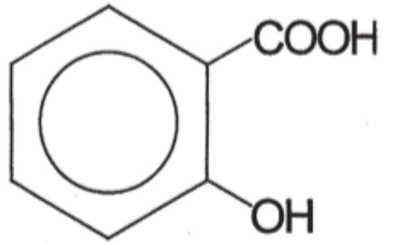
Example

RP-HPLC procedure is developed and validated for the simultaneous quantitation of **aspirin, salicylic acid**, and **caffeine** extracted from an effervescent tablet

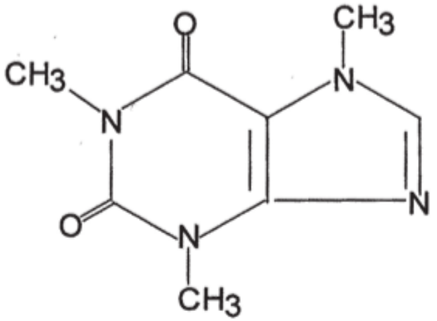
Hypersil C18 column (5 µm, 15 cm × 4.6 mm)
Isocratic elution in a water–methanol–acetic acid
Wavelength of 275 nm



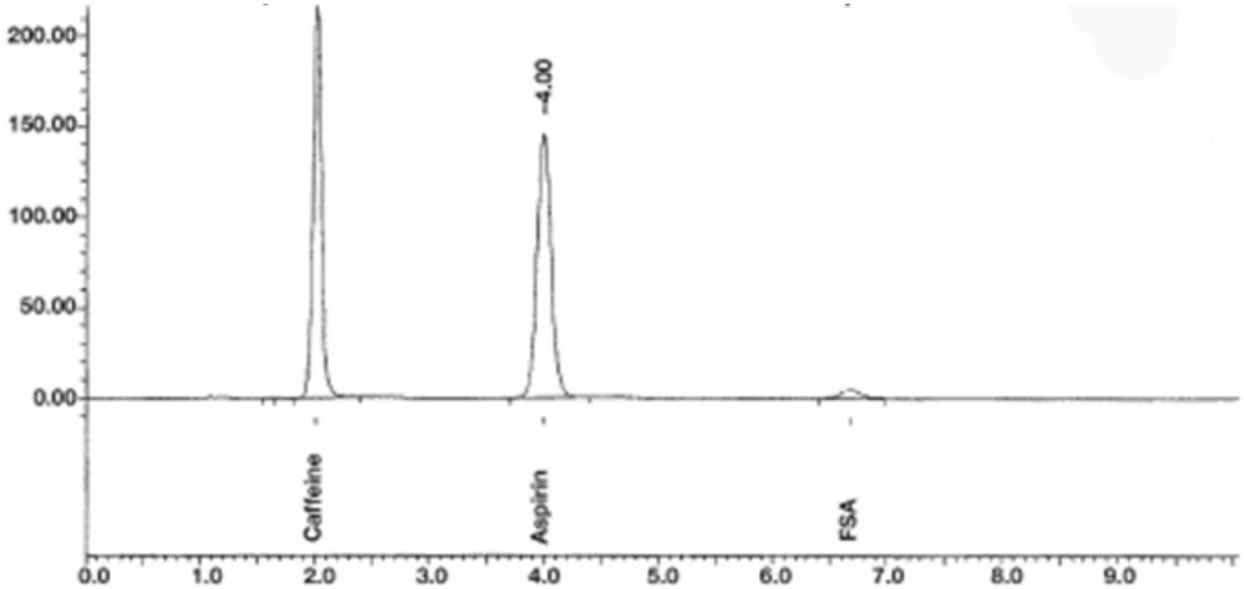
Aspirin



Salicylic acid



Caffeine



Are the three peaks well resolved?

Sawyer, MaryJean, and Vimal Kumar. Journal of chromatographic science 41.8 (2003): 393-397.

Example

Component	%Coefficient of variation ($N = 6$)	%Average recovery (w/w)
Caffeine	1.2	100.1
Aspirin	0.3	99.6
Salicylic acid	2.6	2.0

Which key parameter is the CV(%) related to?

Which key parameter is the Recovery related to?

Sawyer, MaryJean, and Vimal Kumar. Journal of chromatographic science 41.8 (2003): 393-397.

Example

Aspirine

	Nominal percent of 500 mg/tablet				
	50	75	100	125	150
Amount added (mg/mL)	0.5	0.75	1.0	1.25	1.5
Peak area response	635729	944949	1260153	1571021	1833233
Peak area response	636494	950120	1242188	1553390	1816667
Peak area response	633244	946618	1260058	1571866	1818389

Correlation coefficient = 0.99985

R^2 = 99.97%

Caffeine

	Nominal percent of 60 mg/tablet				
	50	75	100	125	150
Amount added (mg/mL)	0.065	0.098	0.130	0.163	0.195
Peak area response	576637	870208	1156438	1440095	1728070
Peak area response	573497	853771	1152395	1440978	1738432
Peak area response	576420	861668	1143538	1431375	1714439

Correlation coefficient = 0.9998

R^2 = 99.98%

Salicylic acid

	Nominal percent at 4% of aspirin (500 mg/tablet)			
	LOQ	4 (100%)	5 (125%)	6 (150%)
Amount added (mg)	0.004	0.100	0.125	0.150
1. Peak area response	5064	50999	63121	74063
2. Peak area response	5029	54007	59775	72196
2. Peak area response	4927	54399	65855	77466

Correlation coefficient = 0.95

Which key parameter they are measuring?
Comment?

Sawyer, MaryJean, and Vimal Kumar. Journal of chromatographic science 41.8 (2003): 393-397.

Example

Table VII. Percent Recovery for Aspirin

	Nominal percent of 500 mg/tablet				
	50	75	100	125	150
Amount added (mg/mL)	0.5	0.75	1.0	1.25	1.5
%Recovery	101.4	100.4	101.3	101.0	98.3
%Recovery	101.5	101.0	99.9	99.9	97.4
%Recovery	101.0	100.6	101.3	101.1	97.5
%Mean recovery Overall = 100.2	101.3	100.7	100.8	100.7	97.7
%RSD Overall = 1.4	0.3	0.3	0.8	0.7	0.5

Table IX. Recovery for Salicylic Acid

	Nominal percent at 4% of aspirin (500 mg/tablet)			
	10	100	125	150
Amount added (mg)	0.004	0.100	0.125	0.150
%Recovery	101.4	100.2	99.3	97.4
%Recovery	100.7	106.2	94.0	94.3
%Recovery	98.7	106.9	103.6	101.7
%Mean recovery Overall = 99.2	100.3	104.4	98.9	97.8
%RSD Overall = 3.4	1.4	2.2	4.8	3.8

Table VIII. Recovery for Caffeine

	Nominal percent of 60 mg/tablet				
	50	75	100	125	150
Amount added (mg)	0.5	0.75	1.0	1.25	1.5
%Recovery	100.7	101.3	101.0	100.6	101.2
%Recovery	100.2	99.4	100.7	101.3	101.8
%Recovery	100.7	100.4	99.9	100.6	100.4
%Mean recovery Overall = 100.7	100.5	100.4	100.5	100.8	101.1
%RSD Overall = 0.6	0.3	0.9	0.6	0.4	0.7

Sawyer, MaryJean, and Vimal Kumar. Journal of chromatographic science 41.8 (2003): 393-397.

Example

Injection	Initial freshly prepared	6 h Room temperature	6 h Chilled	24 h Room temperature	24 h Chilled
Caffeine in standard		100.6%	100.3%	99.3%	99.2%
Caffeine in sample ($N = 3$)	97.2%	96.1%	96.8%	97.0%	96.6%
Aspirin in standard		100.6%	100.3%	99.3%	99.2%
Aspirin in sample ($N = 3$)	100.6%	93.6%	99.7%	70.0%	92.3%
FSA in standard		100.1%	97.7%	97.4%	97.4%
FSA in sample ($N = 3$)	1.7%	7.3%	3.1%	24.5%	7.2%

What they are measuring?

Sawyer, MaryJean, and Vimal Kumar. Journal of chromatographic science 41.8 (2003): 393-397.

Conclusion

- **Pharmaceutical drug analysis ensures the safety, efficacy and quality of medications, supporting public health and regulatory compliance.**
- **A wide array of analytical methods including spectroscopy, chromatography, and hyphenated techniques, address varied drug development challenges.**
- **Focus on identifying, quantifying, and validating drug substances and impurities to meet stringent regulatory standards.**
- **Innovations in analytical technologies continue to enhance drug discovery, development, and monitoring processes.**