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HPLC Method Validation

Introduction

What is Method Validation?

- A systematic process to **demonstrate that an analytical method is suitable** for its intended purpose.
- Required for accuracy, reliability, and regulatory compliance.

Key Objectives of Method Validation

- **Accuracy & Reliability:** Ensures correct measurement.
- **Consistency:** Reliable results across batches and analysts.
- **Regulatory Compliance:** Avoids deficiencies & OOS investigations.
- **Patient Safety:** Ensures drug efficacy and purity.
- **Supports Method Transfer and Global Acceptance:** A validated metho

Current Guidelines for Analytical Method Validation

Regulatory Body	Guideline	Scope
ICH	Q2 (R2)	Global standard for validation of analytical procedures
USP	Chapter <1225>	U.S. Pharmacopeia guidelines on method validation
ANVISA	RE 899	Brazilian Health Regulatory Agency guidelines
AOAC	Official Methods	Standardized methods for food and pharmaceutical analysis
IUPAC	Various Publications	International standards for chemical analysis
FDA (CDER, CBER)	Validation Guidance	U.S. FDA guidelines for pharmaceuticals and biologics

Method Validation

Types of Analytical Methods Requiring Validation

Type of Analytical Method	Purpose	Example Techniques
Identification Methods	Confirms the identity of a drug substance or excipient	FTIR, UV-Vis, Mass Spectrometry (MS)
Assay (Quantitative Tests)	Determines the exact amount of API	HPLC, UPLC, GC, Titration
Impurity Testing	Detects and quantifies impurities or degradants	HPLC, LC-MS, GC-MS, SEC, Ion Chromatography
Dissolution Testing	Measures drug release profile in dissolution media	USP Apparatus I & II, UV-Vis, HPLC
Stability Indicating Methods	Evaluates drug stability under different conditions	Forced Degradation Studies, HPLC, LC-MS
Microbiological Testing	Ensures sterility and microbial limits	ELISA, PCR, Plate Count Method
Content Uniformity	Ensures dose uniformity in pharmaceutical products	HPLC, UV-Vis, Near-Infrared Spectroscopy (NIR)
Residual Solvent Testing	Determines organic volatile impurities (OVIs)	Gas Chromatography (GC), Headspace GC

Each method's validation process typically includes several stages: defining the method's intended use, determining the validation parameters, performing experiments to assess these parameters, and analyzing the results to ensure the method is suitable for its purpose. The regulatory authorities, such as the FDA or EMA, may have specific guidelines for each type of method validation.

Method Validation

Lab Method Flow: Development, Validation, and Transfer

Method development
Establish an **optimized, reliable, and reproducible** analytical method.

Key Steps:
- ✓ Selection of analytical technique (HPLC, GC, UV-Vis, etc.)
- ✓ Mobile phase, column, and detector selection
- ✓ Sample preparation optimization
- ✓ Sensitivity, linearity, and range evaluation

Method Validation
Confirms the method's suitability for its intended use in **routine analysis**.

Key Steps:
- ✓ Specificity
- ✓ Accuracy and Precision
- ✓ Linearity and Range
- ✓ LOD and LOQ
- ✓ Robustness

Method Transfer
Transfers a validated method from one laboratory to another (R&D → QC or site-to-site).

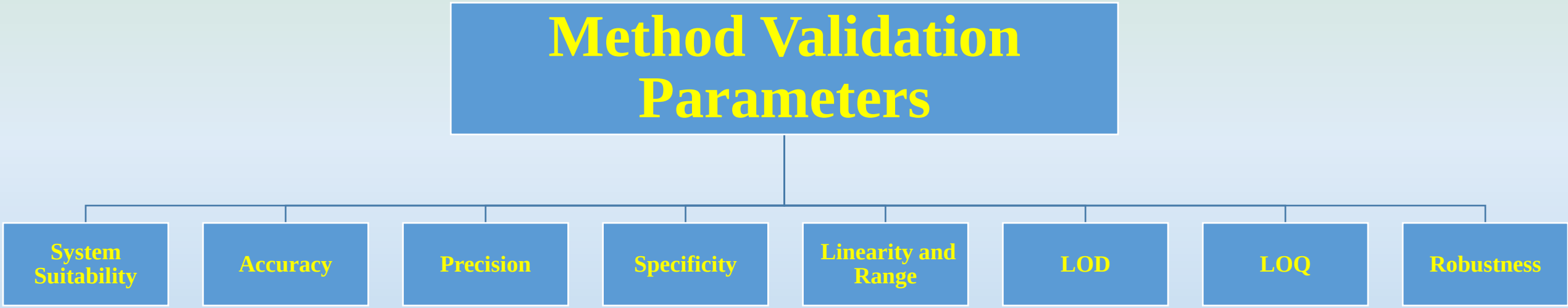
Key Steps:
- ✓ Comparative Testing
- ✓ Co-validation between labs
- ✓ Revalidation at Receiving site

Each phase ensures the method is **fit-for-purpose, robust, and reproducible** before use in quality control or regulatory submission.

Method Validation

HPLC method validation as per **ICH guidelines** ensures consistency, accuracy, and reliability in analytical results. Adhering to these parameters helps maintain product quality and regulatory compliance.

HPLC method validation ensures that an analytical procedure is suitable for its intended purpose. The **International Council for Harmonization (ICH)** outlines specific parameters for validation in **ICH Q2(R2)**.



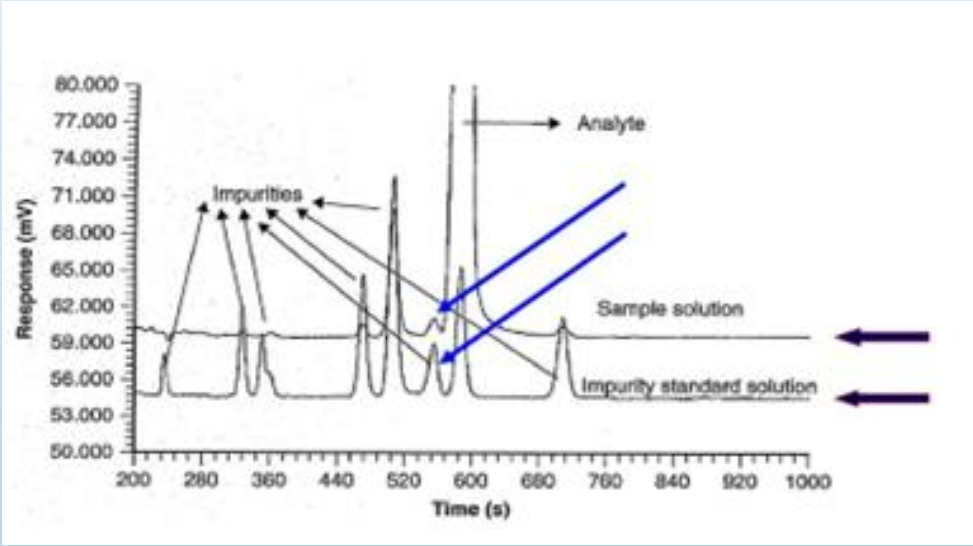
Specificity

Specificity in HPLC-Identification and Interference

Specificity in HPLC refers to the ability of the chromatographic method to unequivocally measure the analyte in the presence of other components, such as impurities, degradation products, or matrix substances. A specific method will only separate and detect the analyte of interest without interference from other compounds.

Assessing Specificity:

- **Chromatogram Quality:**
  Ensure that the analyte peak is well-resolved from other components (impurities, by-products, etc.) with no overlap.
- **Spiking:**
  A known quantity of analyte is added to a sample to confirm the peak observed is indeed due to the target compound.
- **Comparative Studies:**
  Comparing chromatograms from different samples or conditions (e.g., placebo, blank, and spiked samples) helps confirm that the method is specific to the analyte.



Interfering substance

- Active ingredient
- Excipients
- Impurities
- Degradation product

Specificity

Specificity in HPLC-Forced Degradation Study

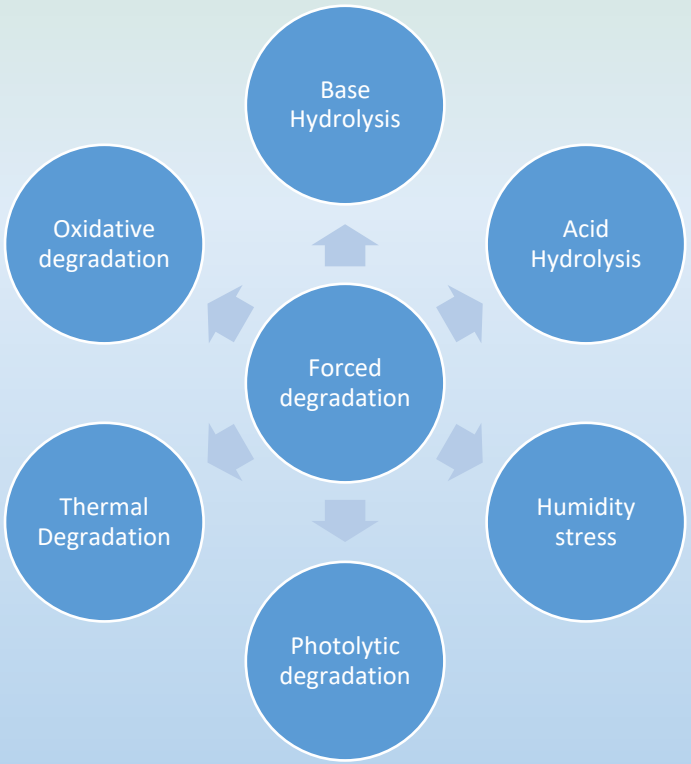
Forced degradation studies (also known as stress testing) are conducted to assess the stability of a drug substance or drug product under extreme conditions. These studies help in the **identification of degradation products**, understanding the degradation pathways, and demonstrating the stability-indicating nature of an analytical method.

Objectives of Forced Degradation Studies

- To establish the **stability-indicating nature** of an HPLC method by ensuring it can separate the active pharmaceutical ingredient (API) from its degradation products.
- To identify possible **degradation pathways** such as hydrolysis, oxidation, photolysis, and thermal degradation.
- To determine degradation kinetics and shelf-life prediction.
- To support formulation development and regulatory submissions.

What we Stress?

- **Drug substance**
- **Drug Product**
- **Placebo**



Specificity

$$\%Degradation = \{100 - \left(\frac{\text{Stressed Sample Peak Area}}{\text{Control Sample Peak Area}} * \frac{\text{Control Sample Weight}}{\text{Stressed Sample Weight}} * 100\right)$$

Stress Condition	Example Conditions	Purpose
Acid Hydrolysis	0.1–1.0 M HCl at 40–80°C for 1–24 hours	Evaluates susceptibility to acidic conditions
Base Hydrolysis	0.1–1.0 M NaOH at 40–80°C for 1–24 hours	Assesses degradation under alkaline conditions
Oxidation	0.3-3% H_2O_2 at room temperature for 1–24 hours	Determines susceptibility to oxidative degradation
Thermal Degradation	50–80°C for weeks/months	Evaluates impact of high temperature
Photolysis (Light Exposure)	Exposure to UV/fluorescent light as per ICH Q1B	Assesses degradation due to light exposure
Humidity	75% relative humidity at 40°C	Evaluates moisture impact on stability

$$Mass\ Balance\ = \frac{(\%Assay\ of\ Stressed\ sample + \sum sum\ of\ impurities\ in\ stressed\ sample}{\%\ Assay\ of\ control\ sample}$$

Acceptance Criteria

- The parent compound should degrade between 5–20% under stress conditions.
- The method is stability-indicating, all degradation products should be well-resolved from the main peak.
- Peak purity should confirm no co-elution of degradants with the API.
- Mass balance should be within 90–110% to ensure all degradants are accounted for.

Specificity

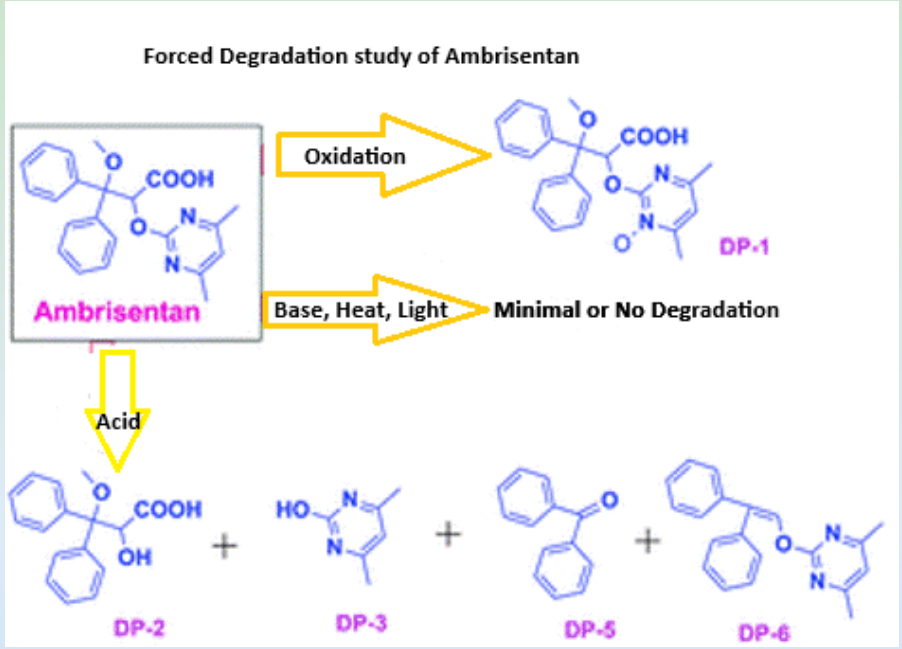
Experimental Approach for Forced Degradation in HPLC:

1. **Preparation of Stock Solution:**
   - ❑ Prepare a standard solution of the drug substance or drug product in a suitable solvent.
2. **Exposure to Stress Conditions:**
   - ❑ Treat the solution with acid, base, peroxide, heat, or light for a predetermined time.
3. **Neutralization (if needed):**
   - ❑ After stress treatment, neutralize the sample (e.g., adding base after acid degradation or vice versa).
1. **Sample Analysis via HPLC:**
   - ❑ Use a validated stability-indicating HPLC method.
   - ❑ Compare stressed samples with unstressed (control) samples.
   - ❑ Check for peak purity using diode-array detection (DAD) or mass spectrometry (MS).
2. **Data Interpretation:**
   - ❑ Identify degradation products.
   - ❑ Assess peak separation and retention time shifts.
   - ❑ Calculate degradation percentage (typically, 5–20% degradation is acceptable to confirm method robustness).

Specificity

Example Results (Hypothetical Data for an API)

Condition	% API Remaining	Major Degradation Products (%)	Observations
Control (Untreated)	100.0%	None	No degradation
Acid (0.1 M HCl, 80°C, 8h)	85.2%	5.3%, 6.5%	Two degradation peaks observed
Base (0.1 M NaOH, 80°C, 8h)	98.9	1.0	Minimal or no degradation
Oxidation (3% H_2O_2, RT, 24h)	92.3%	4.5%, 3.2%	Moderate oxidation
Thermal (70°C, 7 days)	99.1	0.5	Minimal or no degradation
Photostability (ICH Q1B, 1.2 million lux h)	98.1%	1.5%	Minimal light sensitivity



Conclusion

- The method successfully separates the API from all degradation products.
- Acidic and basic conditions led to significant degradation, indicating hydrolysis as a key degradation pathway.
- The method is **stability-indicating**, as all peaks are well-separated with acceptable peak purity.

System Suitability

System Suitability in HPLC

- **System Suitability** in HPLC refers to the process of evaluating and confirming that the HPLC system is operating properly before, during, and after analysis to ensure consistent and reliable results. It involves a set of tests that assess the system's performance and stability under defined conditions, ensuring that the system can produce reliable data for analysis.
- System suitability is a critical aspect of method validation, quality control, and routine laboratory practices, especially in regulated industries such as pharmaceuticals, where accurate and reproducible results are essential.

Key Components of System Suitability in HPLC

- System suitability is assessed by performing tests on several critical parameters of the HPLC system.
- These parameters typically include **retention time, peak resolution, peak symmetry, column efficiency, and reproducibility**.

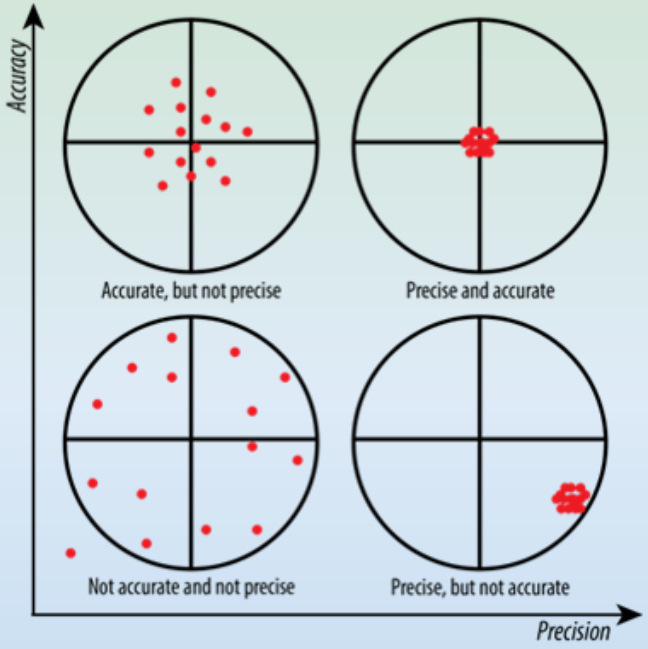
Parameter	Description	Typical Acceptance Criteria
Retention Time (t_k)	The time it takes for a particular compound to pass through the column.	Small variability from run to run is acceptable (typically within ±2%).
Resolution (Rs)	The ability to separate two adjacent peaks.	**Rs ≥ 1.5** for baseline separation (ideal).
Column Efficiency (N)	Number of theoretical plates, representing column performance.	**N ≥ 2000 plates per meter** (depending on column length and analyte).
Tailing Factor (T)	Measures peak symmetry (tailing).	**T ≤ 2** (a value ≤ 1.5 is considered ideal).
Theoretical Plates (N)	Represents column performance in terms of separation efficiency.	**≥ 2000 plates per meter** is typical for a good column.
Signal-to-Noise Ratio (S/N)	Indicates sensitivity of the system to detect small concentrations.	**S/N ≥ 10** is typically required for accurate quantification.
Accuracy and Precision	Variability of the retention times, peak areas, or heights for replicate injections.	**RSD (Relative Standard Deviation) ≤ 2%** for good precision.

Accuracy

Accuracy is the degree of agreement between the test result and the true or accepted reference value. It indicates how close the measured value is to the actual amount of analyte present.

Accuracy can be determined by:

- **Spiking Known Quantities:** Spiking a known quantity of analyte into a blank matrix and comparing the measured value to the true amount.
- **Comparison with a Reference Method:** Comparing the results from the test method with those from a validated reference method.
- **Recovery Studies:** Calculating the percentage of analyte recovered by the assay in a matrix.



Calculation of Accuracy

The accuracy is often expressed as **percent recovery**:

$$Accuracy\ (\%\ Recovery) = \left(\frac{Measured\ Value}{Known\ Value}\right) X\ 100$$

Example Calculation

- Known concentration: 100 µg/mL
- Measured concentration: 98.9 µg/mL

$$Accuracy\ (\%\ Recovery) = \left(\frac{98.9}{100}\right) X\ 100 = 98.9\%$$

Accuracy

Sample Preparation for Accuracy Studies:

- Use at least **3 concentration levels** across the calibration range (e.g., 80%, 100%, and 120% or 50%, 100%, and 150% of the target concentration).
- Prepare **triplicates** for each concentration level to ensure statistical robustness.

Example of Accuracy/Recovery at Assay level

Validation Study	Acceptance Criteria	Results	
Level	Accuracy/Recovery -Assay The individual, mean (n=3) recoveries at each level and overall must be within 97.0 – 103.0%. The %RSD (n=3) at each level and overall (n=9) must be NMT 3.0%.	Sample	%Recovery Sample A
50%		1	99.5
		2	99.9
		3	100.2
		Mean (n=3)	99.9
		%RSD (n=3)	0.45
100%		1	99.4
		2	99.2
		3	100.2
		Mean (n=3)	99.6
		%RSD (n=3)	0.5
150%		1	98.9
		2	99.3
		3	99.8
		Mean (n=3)	99.3
		%RSD (n=3)	0.5
Mean (n=9)			99.6
%RSD (n=9)			0.45

Accuracy

Accuracy/Recovery – Impurity Level

The accuracy and recovery for each known impurity must be evaluated with the following acceptance criteria:

1.Individual and Mean Recovery:

1. The individual and mean recoveries (n=3) at each concentration level should be within the range of **90% to 110%** for each known impurity.

2.Relative Standard Deviation (RSD):

1. The %RSD at each concentration level (n=3) must not exceed **20.0%**.
2. The overall %RSD (calculated from n=9 total replicates) should also not exceed **20.0%** for each impurity.

These criteria ensure the accuracy and precision of the method in recovering impurities at low concentrations.

Precision

Precision

Instrument Precision

Consistency of results when the same sample is injected multiple times under identical conditions.

Method precision (Repeatability)

Consistency of results when the same sample is analyzed multiple times in the same conditions (same day, same operator, same equipment).

Intermediate Precision (Reproducibility within lab)

Consistency of results when the method is applied with variations in instruments, operators, or days.

Inter-laboratory Precision (Reproducibility)

Consistency of results across different laboratories.

Instrument or System Precision

Instrument precision refers to the ability of the HPLC system (including the pump, injector, column, and detector) to deliver consistent results when performing repeated injections of the same sample under identical conditions.

Assessment:
It is typically evaluated by performing multiple injections (e.g., 6-10 injections) of the same standard sample and calculating the **relative standard deviation (RSD)** or **coefficient of variation (CV)** of parameters such as peak area, retention time, or peak height.

Target Value:
For good instrument precision, the RSD of peak area should generally be **<1-2%** for optimal performance. Higher RSD values can indicate issues such as pump fluctuations, poor injector performance, or column degradation.

Example 1: **Multiple Injections of the Same Sample (System Precision)**

Procedure:
Prepare a standard solution of a known analyte (e.g., caffeine or phenol).
1.Inject the same volume (e.g., 10 µL) of the sample into the HPLC system multiple times (e.g., 6-10 injections).
2.Measure the retention time, peak area, and peak height for each injection.

•**Data Analysis**: Calculate the **relative standard deviation (RSD)** of the retention time and peak area from the repeated injections.

$$RSD\ (\%) = \frac{Standard\ Deviation}{Mean\ value}\ X\ 100$$

Instrument or System Precision

Example Result:

Injection	Retention time	Area
1	6.2	3,10,000
2	6.1	3,12,000
3	6.2	3,11,000
4	6.3	3,19,945
5	6.2	3,10,500
6	6.1	3,12,345
Average	6.2	3,12,632
SD	0.1	3,690
RSD	1.2	1.2

•**Interpretation**:

•The **RSD for peak area** is low, indicating excellent precision in the detection system.

•The **RSD for retention time** is also acceptable, indicating that the retention time is reproducible across multiple injections.

Conclusion:

The HPLC system demonstrates good precision, with the observed RSD values being below the generally acceptable thresholds (usually <2%). **Instrument Precision** can be evaluated through multiple injections, column performance, and detector stability to ensure reliable, consistent results.

Method Precision

Repeatability (Intra-day Precision): This measures the variability when the same sample is analyzed multiple times within the same day using the same instrument, operator, and conditions.

Intermediate precision: also referred to as **intra-laboratory reproducibility**, evaluates the consistency of an analytical method when performed under varying conditions within the same laboratory. It ensures that the method remains reliable despite variations in factors such as different days, analysts, instruments, columns, and environmental conditions.

Inter-laboratory precision: also known as **reproducibility**, evaluates how well an HPLC method performs across different laboratories. It ensures that the method remains reliable and consistent when used in different facilities, under varying conditions, and by different operators.

Precision of the method was evaluated by the preparation of six individual sample preparations. Each of the six preparations were injected once per method.

Intermediate Precision

Example to explain Precisions

Repeatability (Intra-day Precision)		Intermediate Precision (Reproducibility within lab)	Inter-laboratory Precision (Reproducibility)
Sample	%Recovery Sample-A1	%Recovery Sample-A2	%Recovery Sample-A3
1	99.2	99.7	99.6
2	99.1	99.2	99.9
3	99.5	100.2	100.1
4	98.6	98.9	99.9
5	98.6	98.8	98.9
6	99.4	99.8	99.4
Mean (n=6)	99.1	99.4	99.6
%RSD (n=6)	0.4	0.6	0.4
Absolute difference (mean)		=A2-A1= 0.3	=A3-A1= 0.5

Typical Acceptance Criteria:

Repeatability (Intra-day Precision):

- %RSD of Six sample be NMT 3.0%.
- For each impurity ≥ 0.05%, the %RSD of the percent impurity results from the (n=6) Sample Solutions is NMT 10.0%.

Intermediate Precision (Reproducibility within lab) and across different laboratories.

- %RSD of Six sample be NMT 3.0%.
- For each impurity ≥ 0.05%, the %RSD of the percent impurity results from the (n=6) Sample Solutions is NMT 10.0%.
- The Difference (absolute) of the mean sample % results between Analyst 1 and Analyst 2 is NMT 3.0.

Range

Range is the interval between the lower and upper concentrations of the analyte where the method demonstrates acceptable accuracy, precision, and linearity. It defines the concentration range over which the HPLC method can provide reliable quantitative results.

Determining Range in HPLC:

- **Lower Limit (LOD - Limit of Detection)**:
  - The lowest concentration of the analyte that can be reliably detected but not necessarily quantified.
- **Upper Limit (ULOQ - Upper Limit of Quantification)**:
  - The highest concentration of the analyte that can be measured with acceptable accuracy and precision.
- **Practical Range**:
  - The concentration range between the LOD and ULOQ, where the calibration curve is valid, and the method is both linear and accurate.

How to Establish Linearity and Range:

1.Prepare Standards:
- Prepare multiple standard solutions of the analyte at different concentrations.

2.Measure Response:
- Inject the standards into the HPLC system and record the peak area (or height).

3.Construct Calibration Curve:
- Plot the concentration vs. peak response and fit a linear regression line.

4.Evaluate Linearity:
- Ensure the correlation coefficient r2 is close to 1 (typically ≥ 0.999).

5.Define Range:
- Confirm the method's performance at the lower and upper limits by checking accuracy, precision, and recovery across the concentration

Linearity

Linearity:

Linearity refers to the ability of the HPLC method to produce results that are directly proportional to the concentration of the analyte over a given range. A linear relationship means that as the concentration of the analyte increases, the signal (e.g., peak area or height) also increases proportionally.

Key Aspects of Linearity in HPLC:

•Determined by:

A calibration curve, which plots the analyte concentration (x-axis) against the corresponding signal (peak area or height) on the y-axis.

•Equation for Linearity:

A linear regression equation is typically used to represent the calibration curve:

$$y=mx+b$$

Where:

- y is the signal (e.g., peak area or height),
- x is the concentration of the analyte,
- m is the slope of the line (sensitivity),
- b is the intercept (should ideally be zero for pure linearity).

•Verification:

The **correlation coefficient (r^2)** is used to assess the degree of linearity. An **r^2 value close to 1** indicates excellent linearity.

Linearity

Prepare Standard Solutions:

To assess linearity, you need to prepare several standard solutions with varying known concentrations of the analyte. These should cover the expected range of concentrations for the method.

- **Concentrations should span the expected range**, from the **Limit of Detection (LOD)** to the **Upper Limit of Quantification (ULOQ)**.

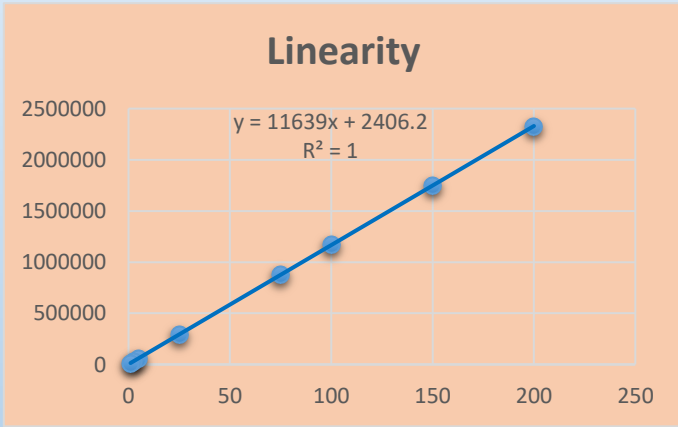
- For best results, it's common to use 5–7 standard solutions at different concentration levels (low, medium, high).

Standards	Concentration	HPLC area
LS1 (ULOQ)	200	2328072
LS2	150	1746048
LS3	100	1170406
LS4	75	880007
LS5	25	294581
LS6	5	59060
LS7	2.5	29664
LS 8 (LOQ)	1	11922
Intercept		2406
Slope		11639
r^2		1

Calculate the Correlation Coefficient (r^2)

The **correlation coefficient (r^2)** quantifies how well the data fits a straight line. It ranges from 0 to 1:

- **$r^2 = 1$**: Perfect linear relationship (ideal scenario).
- **$r^2 \geq 0.999$**: Generally accepted for reliable linearity.
- **$r^2 < 0.99$**: Indicates poor linearity, suggesting the need for method optimization.



LOD and LOQ

LOD (Limit of Detection) and LOQ (Limit of Quantification) in HPLC

LOD and **LOQ** are key parameters in HPLC that define the lowest levels at which an analyte can be reliably detected and quantified, respectively. These limits are crucial for method validation, particularly when detecting trace amounts of compounds in samples.

Limit of Detection (LOD)

The **LOD** is the lowest concentration of an analyte that can be detected but not necessarily quantified with reasonable certainty. It is the point where the analyte's signal is distinguishable from the background noise, but the measurement may not be precise enough to quantify the concentration accurately.

LOD Calculation:

One common method for determining LOD is based on the **signal-to-noise ratio (S/N)**. It is often defined as the concentration at which the signal-to-noise ratio is ≥ **3:1** (3 times the standard deviation of the baseline noise).

$$LOD = \frac{3\ X\ Standard\ deviation\ of\ the\ Noise}{Slope}$$

Typical LOD Criteria:

- **S/N ≥ 3**: The analyte signal is distinguishable from noise but not yet quantifiable.
- LOD is typically **lower than LOQ** and is more useful in qualitative analysis or screening applications.

LOD and LOQ

Limit of Quantification (LOQ)

The **LOQ** is the lowest concentration of an analyte that can be reliably quantified with acceptable accuracy and precision. At this concentration, the analyte's signal can not only be detected but also quantified with an acceptable degree of confidence.

LOQ Calculation:

A common method for calculating LOQ is based on the **signal-to-noise ratio (S/N)**, where a ratio of **S/N ≥ 10** is used. It can also be calculated using the following formula:

$$LOD = \frac{10\ X\ Standard\ deviation\ of\ the\ Noise}{Slope}$$

Typical LOQ Criteria:

- **S/N ≥ 10**: The analyte's concentration is quantifiable with good precision and accuracy.
- **Precision**: Relative standard deviation (RSD) at LOQ should be acceptable (typically ≤ 20%).
- **Accuracy**: The measured concentration at LOQ should be within ±20% of the true concentration.

LOD and LOQ

Establishing LOD and LOQ in HPLC:

1. **Inject Low Concentration Samples**:
   - Prepare samples with low concentrations of the analyte and inject them into the HPLC system.
2. **Measure Baseline Noise:**
   - Measure the noise level in blank samples (no analyte) to calculate the signal-to-noise ratio.
3. **Determine Detection**:
   - For LOD, determine the point at which the analyte's signal is distinguishable from the baseline noise ($S/N \geq 3$).
4. **Determine Quantification**:
   - For LOQ, determine the lowest concentration where the analyte's signal can be reliably quantified with $S/N \geq 10$.
5. **Check Precision and Accuracy**:
   - At the LOQ, ensure that the precision (RSD) and accuracy (bias) meet the required criteria for your application.

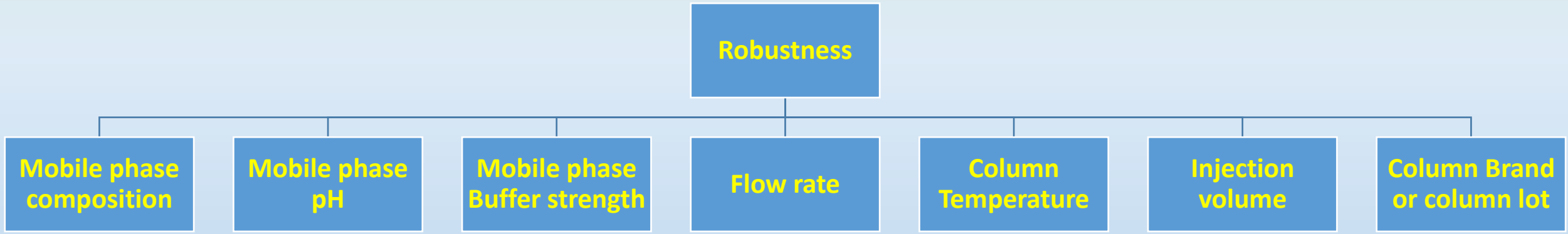
For pharmaceutical and chemical analysis (e.g., ICH guidelines), both **LOD** and **LOQ** need to be validated as part of **method validation** to ensure the HPLC method is capable of detecting and quantifying trace amounts of the analyte in real-world samples, particularly when dealing with impurities, degradation products, or low-concentration active ingredients.

Robustness

Robustness in HPLC

Robustness in HPLC refers to the ability of a chromatographic method to remain unaffected by small, deliberate variations in method parameters, while still producing reliable and consistent results. It assesses the method's **reliability** and **resilience** to changes in operational conditions.

A robust HPLC method is essential for routine analysis, as small changes in system parameters such as temperature, pH, flow rate, and column conditions are inevitable during regular use. The method must be able to handle these variations without significant loss in performance.



Robustness

Evaluate Performance

- **Retention Time**: Check if retention times change significantly.
- **Resolution:** Verify that the resolution between peaks remains acceptable.
- **Peak Shape:** Ensure that the shape of the peaks does not become distorted (e.g., no tailing or fronting).
- **Reproducibility:** Ensure that the method's precision (relative standard deviation, RSD) is within acceptable limits.
- **Resolution and Efficiency:** Check if changes in these parameters affect the separation quality or column efficiency.

Robustness	Nominal	Altered
Mobile phase composition	Mobile Phase: %A: %B (50:50)	Mobile Phase: %A: %B (55:45) %A: %B (45:50)
Mobile Phase pH	5.0	4.8 and 5.2
Buffer strength	50 mM	48 mM and 52 mM
Flow rate	1.0 ml/Min	0.9 ml/min and 1.1 ml/min
Column Temperature	40 ^{0}C	38 ^{0}C and 42 ^{0}C
Injection Volume	50 µl	45 µl and 55 µl
Column Brand	Phenomenex	Agilent
Column Lot#	Phenomenex Column Lot#1	Phenomenex Column Lot#2

Data Analysis

- **Compare chromatograms** from all variations to determine whether the method still meets its acceptance criteria (e.g., resolution, peak symmetry, efficiency).
- **Statistical Analysis**: If necessary, perform a statistical analysis (e.g., ANOVA) to assess the significance of the variations and determine whether they impact the method's performance.
- **%Difference** in between the average from Precision (nominal condition) and each varied condition must be NMT ± 2.0 for assay of sample

Revalidations

Revalidation is essential to confirm that the method still provides reliable, accurate, and reproducible results under the new conditions. The level of revalidation (whether partial or full) depends on the nature of the changes and their potential impact on the analytical method's performance.

- **Change in Analytical Procedure**:
  Modifications in the method (e.g., change in equipment, reagents, or test conditions)
  Changes in procedures like extraction methods, calibration techniques, or detection limits
- **Change in Drug Substance:**
  Alterations in the chemical composition or physical properties of the drug substance (such as polymorphs, impurities, or salt forms)
- **Change in Drug Product:**
  Formulation changes (e.g., excipient changes, strength modifications, or packaging)

Validation Report format

Generally method validation report shall have (but not limited to)

1. **Title Page**:
   - Includes the method name, date, and version number.
2. **Objective:**
   - States the purpose and scope of the validation,
3. **Method Description:**
   - A detailed description of the analytical method, including the instrumentation, reagents, standards
4. **Validation Criteria:**
   - Lists the parameters to be validated (e.g., specificity, accuracy, precision, linearity, range, detection limit, etc.).
5. **Experimental Conditions:**
   - Details of the conditions under which the validation studies were performed,
6. **Results:**
   - Presents the validation data, often in tables or graphs, with a detailed discussion of the results
7. **Discussion:**
   - Interpretation of results, comparison to acceptance criteria, and an evaluation of the method's performance.
8. **Conclusion:**
   - Summary of whether the method meets the acceptance criteria and is suitable for its intended use.
9. **References:**
   - Citations to any relevant guidelines (e.g., ICH, USP, FDA), previous validation studies, or other supporting documents.
10. **Appendices:**
    - Includes any additional information such as raw data, calculations, chromatograms, or specific protocols

Validation Report: Example

Company Logo or Name	METHOD VALIDATION REPORT	
Report Number	Release Date:	Page 1 of 2
Title:		

Title: Report on the Validation of the Identification and Determination of Assay product A in tablet (X.X% w/w) by HPLC

Report Number
Method Validation Report-XXX

Method Number
XXX

Project Number
XXX

Testing Facility
Company Name and address

Sponsor
Company name and address

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Company Logo or Name	METHOD VALIDATION REPORT	
Report Number	Release Date:	Page 2 of 2
Title:		

Conclusion

In conclusion, the validation of the analytical method has been successfully completed, demonstrating that the method meets all required criteria for its intended use. The validation process involved evaluating the key parameters as outlined by regulatory standards (e.g., ICH, USP), ensuring that the method provides reliable, accurate, and precise results.

Key Findings:

- **Accuracy**: The method provided results that are in close agreement with known reference values, confirming its accuracy.
- **Precision**: The method showed consistent and reproducible results across different days and operators, proving its reliability.
- **Specificity**: The method is specific for the target analyte(s), with no significant interference from other sample components.
- **Linearity**: A clear linear relationship between analyte concentration and detector response was established within the specified range.
- **Range**: The method proved effective across a broad range of concentrations, without significant deviation in performance.
- **LOD & LOQ**: The method is sensitive enough to detect and quantify low levels of the analyte, as confirmed by LOD and LOQ testing.
- **Robustness**: The method demonstrated robustness, maintaining performance despite minor changes in operational conditions (e.g., temperature, pH).
- **System Suitability**: The system met all predefined criteria, ensuring that it is operating within specifications during routine analysis.

Final Statement:

The validated method is now confirmed to be suitable for its intended purpose, ensuring accurate, reliable, and reproducible results for its application in **quality control, regulatory compliance, and other relevant areas**. Regular monitoring and periodic revalidation are recommended to ensure continued method performance over time.

THANK YOU