Analytical methods

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2. Specifications

Outline of the Presentation

1. Objectives and expected outcomes
2. Specifications
3. The Rational for Analytical methods development
4. Analytical Methods
5. Analytical method validation/verification

1. Objectives and expected outcomes

- At the end of the presentation participant shall be able to understand
  - analytical methods,
  - analytical method validation/verification
- At the end of the presentation participant should also be able to
  - Properly validate Analytical Methods
  - review and identify **deficiency/ies** with regard to analytical method.

Specifications

What is Specification?

- A specification is defined as a list of tests, references to analytical procedures, and with appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described.
- It establishes the set of criteria to which a drug substance or drug product should conform to be considered acceptable for its intended use

Ser. No	Test Type	Specifications Limit	Analytical Procedures
1	Description		SOP---
2	Identification		USP----
3	Assay		USP----

Specification...contd

- Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval, for MA purpose
- Specifications are chosen to confirm the quality of the drug substance and drug product.

Setting Specification

- When a specification is first proposed, It should be justified:
- The justification should refer:
  - to relevant Pharmaceutical development data,
  - Pharmacopoeial standards,
  - test data for drug substances and drug products used in toxicology and clinical studies,
  - results from accelerated and long term stability studies,
  - A reasonable range of expected analytical and manufacturing variability should be considered.
  - It is important to consider all of this information.

- Therefore, properly Developed and Validated Analytical Method is required to test a drug substance or drug Product against the Specification Set

3. The Rational For Analytical method development

Analytical Method Development……ctd

- Analytical method development plays important role in the discovery, development, and manufacturing of pharmaceuticals.
- The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one.
- Therefore, it becomes necessary to develop newer analytical methods for such drugs.
- The official analytical methods are results of these processes
  - are used by the manufactures and quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

Basic reason for new method development :-

- The drug or drug combinations may not be official in any pharmacopoeias,
- A proper analytical procedure for the drugs may not be available in the literature due to patent rights,
- Analytical methods may not be available for the drug in the form of a formulation.

Basic reason……ctd

- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents.
- It may also involve extraction and separation procedures and these may not be reliable.

Basic reason……ctd

- Existing methods may have error, artifact , or contamination prone, or they may be unreliable (have poor accuracy or precision).
- Existing methods may not provide adequate sensitivity or analyte selectivity in samples of interest. Example, method used for cleaning procedures.

Basic reason……ctd

- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including:
  - improved analyte identification or detection limits,
  - greater accuracy or precision, or better return on investment

5.Analytical Methods

Analytical Methods

- Instrumental or non instrumental methods that are used for:
  - Determination of potency/assay, which can directly relate to a known dose or formulation strength/concentration
  - Determination of impurities, which can relate to the safety profile of the drug
  - Evaluation of degradation products/stability profile of pharmaceutical products

Analytical Methods cont…

- Evaluation of key drug characteristics such as crystal form, drug release, drug uniformity, properties which can compromise bioavailability
- Evaluation of key manufacturing parameters/Process, to ensure that production of drug substances/ drug products is consistent.

Types of Analytical Methods

A. Regulatory Analytical Methods

- Are analytical Procedures found in pharmacopeias of different countries (Examples: USP, BP, EP, JP and so on)
- A regulatory analytical methods is the analytical procedure used to evaluate a defined characteristic of the drug substance or drug product which are accepted by the authority

B. Alternative Analytical Procedure

- An alternative analytical procedure is an analytical procedure proposed by the applicant for use instead of the regulatory analytical procedure.
- A validated alternative analytical procedure should be used only if it is shown to perform equal to or better than the regulatory analytical procedure.

Alternative Analytical Procedure cont..

- If an alternative analytical procedure is used, the manufacture should have/provide:
  - A rationale for its inclusion and identify its use (e.g., release, stability testing),
  - Validation data,
  - Comparative data to the regulatory analytical procedure.

C. (None) Stability Indicative Method

- A stability-indicating assay is a validated quantitative analytical procedure that can detect the changes with time in the properties of the drug substance and drug product.
- A stability-indicating assay accurately measures the active ingredient(s), without interference from:
  - degradation products,
  - process impurities,
  - excipients,
  - or other potential impurities.

Stability Indicative Method cont…

- If a manufacturer uses a non-stability-indicating analytical procedure for release testing,
  - then an analytical procedure capable of qualitatively and quantitatively monitoring the impurities, including degradation products, should complement it.
- Assay analytical procedures for stability studies should be stability-indicating

6. Analytical Method Validation/Verification

Method Validation

Method validation can be defined as:

- Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.
- It is the process of demonstrating that analytical procedures are suitable for their intended use.

Content and format of analytical procedures

- Any analytical procedure:
  - should be described in sufficient detail to allow a competent analyst to reproduce the necessary conditions and obtain results comparable to the applicant.
- .

Content and format of analytical procedures cont…

- The following is a list of information that should typically be included in a description of an analytical procedure.
  - ✓ Principle
  - ✓ Sampling and Preparation of Samples
  - ✓ Equipment and Equipment Parameters
  - ✓ Reagents
  - ✓ Preparation of Standards
  - ✓ Procedure
  - ✓ System Suitability Testing
  - ✓ Calculations
  - ✓ Reporting of Results
  - ✓ Qualitative and quantitative limits

Compendial methods-Verification

- All methods are appropriately validated as specified under *Validation of Compendial Procedures (1225).*
- *A validated method may be used to test a new formulation only after confirming that the new formulation does not interfere with the accuracy, linearity, or precision of the method (i.e. Verification).*
- It may not be assumed that a validated method could correctly measure the active ingredient in a formulation that is different from the one used in establishing the original validity of the method
- Therefore, assay method for APIs may not be required?!

Compendial methods….ctd

- Compendial methods should be verified for its proper performance especially for dosage forms.
- The parameters to be verified for formulations depends on:
  - Nature of Matrices,
  - Intended applications,
  - USP general chapter <1226> VERIFICATION OF COMPENDIAL PROCEDURES

Compendial methods…..ctd

- When a manufacturer claims/uses a compendial method, there should be no change in:
  - The type of column i.e the stationary phases
  - Detector wavelength
  - Components in Mobile phase
  - System suitability testing and criteria

Compendial methods……ctd

- ratio of components in mobile phase
- flow rate
- column temp, dimension of column, particle size
- Adjustments to: Eg USP general chapter <621>.

- Full validation is required for specified impurities that are not included in the monograph
  - specificity, linearity, accuracy, repeatability, intermediate precision, LOD/LOQ
- *What if there is change from these Conditions?*

Non-compendial methods-Validation

- Full validation is required for these type of methods
- Performance characteristics are discrete attributes, obtained by actual execution of an analytical method using controlled samples are used for method validation.
- Demonstrate the suitability and proper use of the method for a specific application

  - Specificity
  - Accuracy
  - Linearity
  - Precision
  - LOD/LOQ
  - Robustness

Pre-validation Works

- Equipment: Suitable for expected task?
- Reference Materials and reagents: Suitability
- Personnel's: Is trained and qualified
- Analytical Method: Is procedure finalized?
- Validation Protocol: Management / Approval

Validation Protocols

- The validation Protocol document should at. least contains:
  - Description of the analytical method and its intended use (purpose)
  - Lists of instruments/apparatus
  - Personnel competency
  - List of applicable performance characteristics
  - Appropriate acceptance criteria
  - Review and approval date/signatures

Table: Test parameters with acceptance criteria

Ser. No.	Parameter	Acceptance Criteria
1	Linearity	
	• Correlation coefficient	> 0.99
	• Y-intercept (relative to the 100% calculated value)	±2.0%
	• Visual	Linear
2	Accuracy	Percent Recovery
	• Recovery of each over the whole range	96.0–104.0%
	• Mean recovery per concentration	98.0–102.0%
	• RSD (n=9)	≤2.0%
3	Precision	RSD
	• System Repeatability	≤1.0%
	• Method Repeatability	≤2.0%
	• Intermediate Precision	Pooled RSD ≤3.0% + Absolute mean assay difference ≤ 3.0%
4	Specificity	
	• using DAD	Peak Purity Index ≥ 0.999
5	Robustness	Not to affect the system suitability criteria
6	LOD and LOQ	To report the calculated values
7	Sample Solution Stability	RPD ≤ 2.0%
8	Filtration Study	RPD ≤ 2.0%

Performance Characteristics cont…

Validation Characteristics	Identification	Testing for Impurities		Assay, Dissolution (measurement only), Content/Potency
		Quantitative	Limit	
Accuracy	-	+	-	+
Precision			-	
Repeatability	-	+	-	+
Interm. precision	-	+	-	+
Specificity	+	+	+	+
Detection limit	-	-	+	-
Quantitation limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+
Robustness	-	+	-	+

1.Specificity

- The ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components

- Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s)

- Specificity is concentration-dependent and should be determined at the low end of the calibration range.

Specificity Cont…

Specificity Applied

- Identification
  - to ensure the identity of the analyte
- Purity Test
  - accurate statement of the content of impurities of an analyte (related substances, residual solvents, etc.)
- Assay
  - an exact result which allows an accurate statement on the content of potency of the analyte in a sample

Specificity Cont..

- Some analytical procedures are not sufficiently specific for the intended purpose
  - Assay by titration
  - Assay of enantiomer by HPLC method
  - Identification by UV absorbance
- A combination of two or more analytical procedures is recommended to achieve sufficient specificity under such condition

Specificity Cont..

- When the criteria are not met, this indicates that the method is not sufficiently developed
- Poor specificity can impact accuracy, precision and linearity

Samples for Specificity Testing

- Blank solution to show no interference with any HPLC system artifact peak.
- Placebo to demonstrate the lack of interference from excipients.
- Drug substance to show that all other substances are resolved from the drug substance.
- Authentic samples of critical related substances to show that all known related substances are resolved from each other

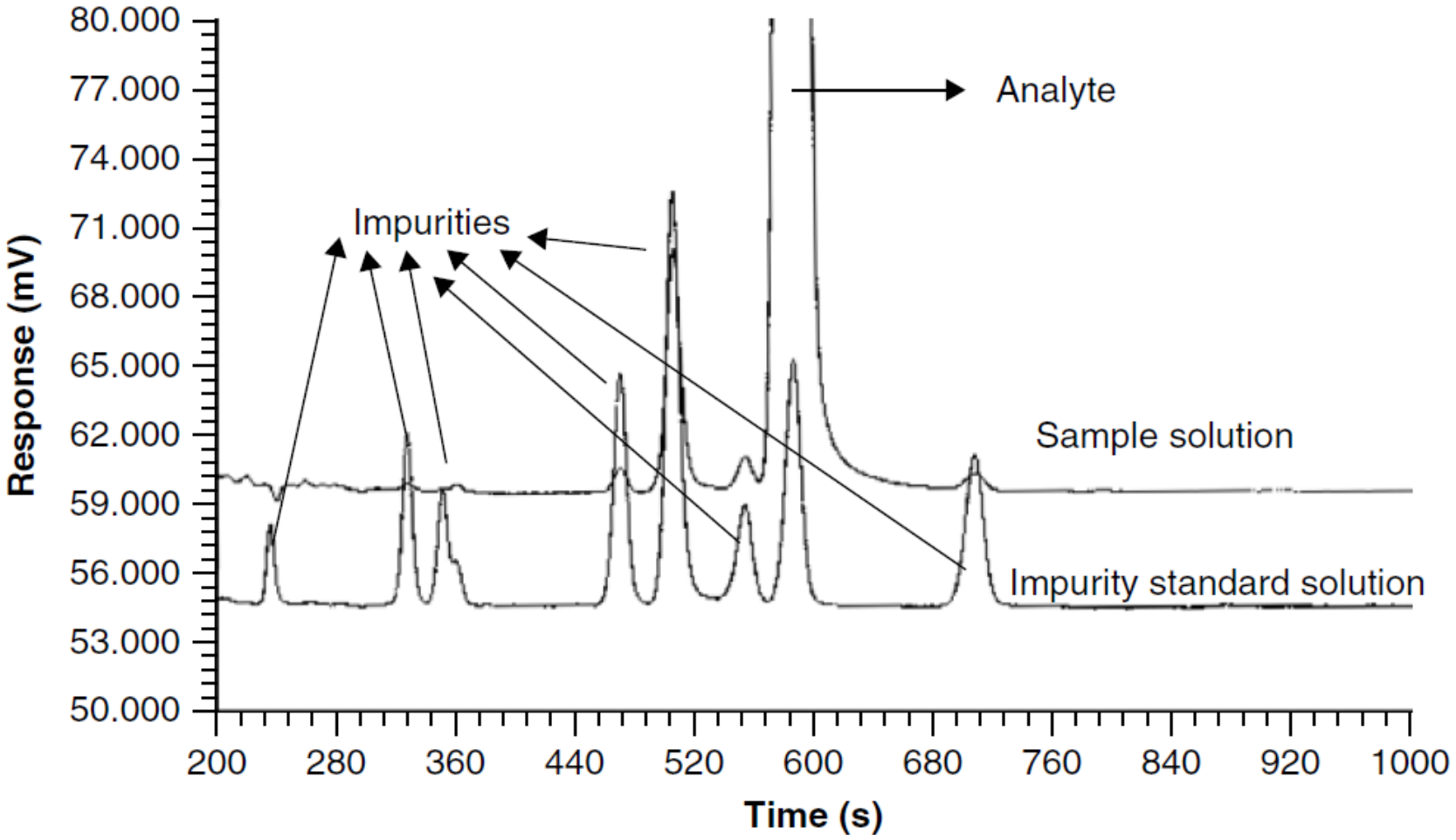
How Specificity?

- The specificity of a test method is determined by comparing test results from an analysis of samples containing impurities, degradation products, or placebo ingredients with those obtained from an analysis of samples without impurities, degradation products, or placebo ingredients.

- For the purpose of a stability indicating assay method, degradation peaks need to be resolved from the drug substance. However, they do not need to be resolved from each other.

- Specificity can best be demonstrated by the resolution of two chromographic peaks that elute close to each other. In the potency assay, one of the peaks would be the analyte peak.

Example,


80.000
77.000
74.000
71.000
68.000
65.000
62.000
59.000
56.000
53.000
50.000
Response (mV)
200
280
360
440
520
600
680
760
840
920
1000
Time (s)
Analyte
Impurities
Sample solution
Impurity standard solution

Approaches

1. *When authentic samples of related substance are available.*

- Analyze stressed drug product, placebo, drug substance, stressed placebo, and solutions spiked with authentic samples of related substances.
- The HPLC chromatograms are used to show the resolution among related substances, drug substance, and other potential interferences.

Approaches……

2. When authentic samples of impurities are not available.

- *A stressed drug product* can be analyzed to show separation of the most significant related substances
- The peak homogeneity of the stressed sample should be investigated by PDA or mass spectrometry
- Typically, a stressed sample of about 10 % to 20% degradation is used. A 10 to 20% degraded sample is used because it has a sufficiently high concentration level of critical related substance.

2. Linearity

- Linearity is the ability of analytical procedure to produce test results which are proportional to the concentration (amount) of analyte in samples within a given concentration range.
- Justifies the use of a single point standard to quantitative across the range
- Performed directly on the drug substance (dilution of standard stock solution)
- Minimum of five concentration is recommended from 80% to 120 % of the analytical concentration

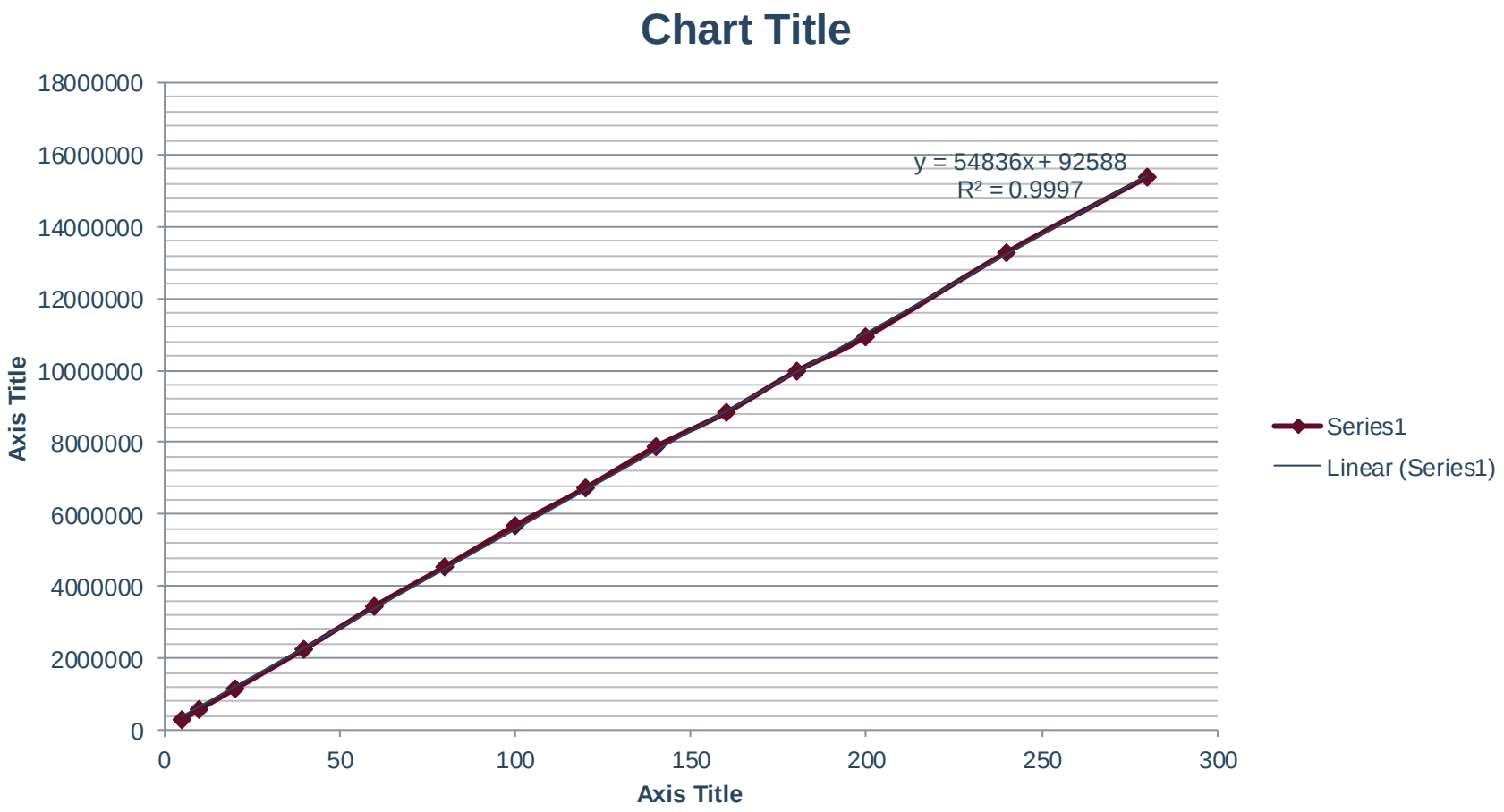
Linearity Cont…

Validation Characteristics	Principles	Experimental condition	Acceptance criteria
Linearity	Direct relation of response with concentration	• Prepare standard stock solution (different weighing of different substance)	• A plot of the data should be reported
		• Prepare serial dilution (5-6) from stock solution (80%-120%) of the expected conc.	• The slope of the line , intercept and correlation coefficient (>/=0.99)

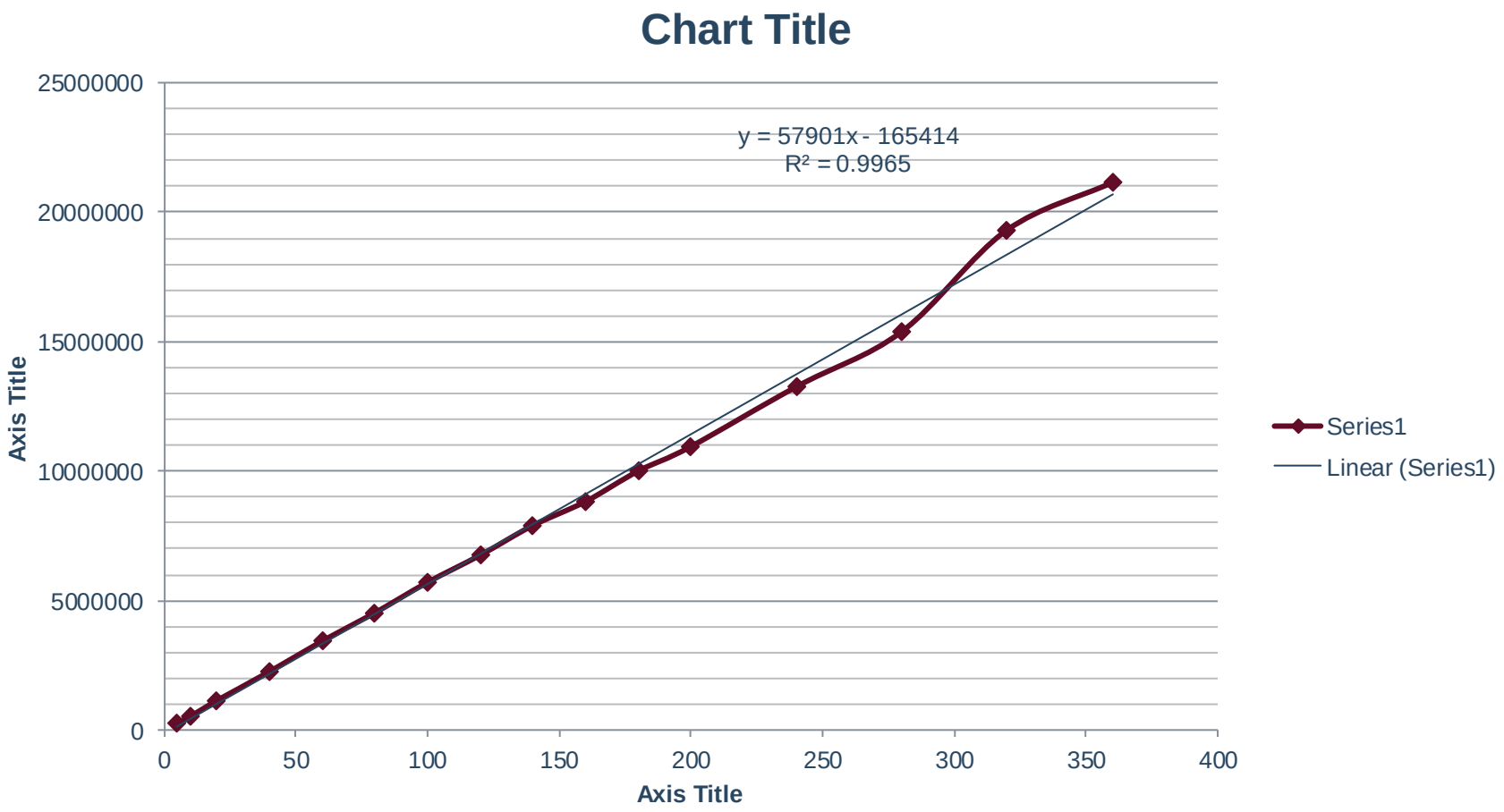
Linearity, practical example

Calibration Levels	**Concentration (micgr/mL)**	**Response (Peak Area)**	Response factor
1	5	301568	60313.6
2	10	567413	56741.3
3	20	1126639	56332.0
4	40	2248305	56207.6
5	60	3421719	57028.7
6	80	4544013	56800.2
7	100	**5702036**	57020.4
8	120	6743868	56198.9
9	140	7885679	56326.3
10	160	8846651	55291.6
11	180	10002721	55570.7
12	200	10915064	54575.3
13	240	13286836	55361.8
14	280	15360306	54858.2
15	320	19269779	**56024.1**
16	360	21170824	**58807.8**
			1.8

Linearity, practical example (Excluding the reds)



Linearity, practical example (Including the reds)



3. Accuracy

- The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value.

- ICH recommends a minimum of 9 determinations over a minimum of 3 concentrations covering the stated quantitation range (3 test at 3 conc.) (Ex. For Assay: 80, 100 and 120%)

Accuracy…….

- For a drug substance, the common method of determining accuracy is to apply the analytical procedure to the drug substance and to quantitate it against a reference standard of known purity.
- For the drug product, accuracy is usually determined by application of the analytical procedure to synthetic mixtures of the drug product components or placebo dosage form to which known quantities of drug substance of known purity have been added
- *Experimental Considerations.*

➢ *Typically, known amounts of related substances* and the drug substance in placebo are spiked to prepare an *accuracy sample of* known concentration of related substance and drug product

Accuracy Cont…

Acceptance criteria

% Active/impurity content	Acceptable mean recovery
≥ 10	98 –102%
1 -10	90 –110%
0.1 – 1	80 – 120%
< 0.1	75 – 125%

Table Recovery of D1 and D2 three concentration levels 50 %, 100 % and 150 % of the analytical concentration in triplicate preparations at each level

Levels	Average peak area spiked sample		Average peak area of standard recovered		Amount add (mg)		Amount recovered (mg)		Percent recovered	
	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
50 % - 1	3585970.0	7113609.0	1333420.3	2353278.9	2.0015	2.0027	2.0067	2.0349	100.3	101.6
50 % - 2	3615666.0	7162589.0	1351445.1	2377594.0	2.0015	2.0027	2.0344	2.0552	101.6	102.6
50 % - 3	3642196.5	7170121.5	1354633.1	2335796.7	2.0015	2.0027	2.0393	2.0203	101.9	100.9
100 % - 1	6252466.0	12056729.5	4011587.6	7321064.3	6.0044	6.0080	6.1114	6.1811	101.8	102.9
100 % - 2	6207407.5	12050038.5	3919844.1	7215713.7	6.0044	6.0080	5.9708	6.0932	99.4	101.4
100 % - 3	6207264.0	11942704.0	3954714.3	7182373.9	6.0044	6.0080	6.0243	6.0653	100.3	101.0
150 % - 1	9349575.0	17734261.0	7132039.0	13047925.7	11.0080	11.0147	10.8910	10.9608	99.0	99.5
150 % - 2	9509192.5	17917261.0	7256642.8	13156930.9	11.0080	11.0147	11.0850	11.0518	100.7	100.3
150 % - 3	9404765.5	17781983.0	7163887.1	13046317.8	11.0080	11.0147	10.9428	10.9595	99.4	99.5

4. Precision

- The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.
- Determination
  - Assay individual samples of a homogeneous preparation
  - Calculate Standard Deviation or Relative Standard Deviation
- Precision is expressed as RSD

Precision Cont…

Level of precision

A. Repeatability

- Agreement within a short period of the same analyst and instrumentation
- System and method precision
- n=6, 100% level

B. Intermediate precision

- Agreement in results intra-laboratory but from different days, analysts and equipment (as appropriate)

C. Reproducibility

- Agreement in results between laboratories (as in a collaboration study)…..(If C is done, B is not required)

Precision Cont…

Acceptance criteria (Repeatability)

Component measured in sample	Precision (in RSD)
>10.0%	≤ 2%
1.0 up to 10.0%	≤ 5%
0.1 up to 1.0%	≤ 10%
< 0.1%	≤ 20%

:

Table: System precision data of performed at the analytical concentration (n=6) of the standard solutions

Ser. No.	Peak area of D1	Peak area of D2
1	6497180	11697872
2	6468308	11682527
3	6530359	11756621
4	6484146	11739370
5	6475588	11756981
6	6494368	11741426
Average	6491658.2	11729132.8
SD	21888.4	31421.0
RSD	0.3	0.3

Table : Method Precision data of method validation performed at the analytical concentration on multiple preparation (n=6) of the same homogenous sample

Replication	Average peak area	
	D1	D2
Preparation 1	7559824.6	16089877
Preparation 2	7542248.4	15949232
Preparation 3	7693793.2	16438709
Preparation 4	7454508.2	15896539
Preparation 5	7371967.2	15913313
Preparation 6	7525189.9	16243777
Average	7524588.6	16088574.5
SD	120818.2	226268.0
RSD	1.6	1.4

Precision Cont…

- Intermediate Precision
- FDA recommends a minimum of 2 different days with different analysts
- Acceptance criteria
  - Perform F-test
  - results between analysts should not be statistically different
  - Typically about 2x repeatability (2 x RSD), or pooled RSD and Absolute mean difference

Table Intermediate Precision data of method validation performed by multiple analyses (n = 6) of the same homogenous sample by two analysts on two different days, using independently prepared reagents and sample preparations, and on two different equipment setups

Ser No.	Percent Recovered (n=6) Day I & Analyst I		Percent Recovered (n=6) Day II and Analyst II	
	D1	D2	D1	D2
1	115.5	96.4	115.9	98.2
2	115.2	95.6	115.7	98.6
3	117.6	98.5	116.0	98.4
4	113.9	95.3	116.7	98.9
5	112.6	95.4	115.2	98.5
6	115.0	97.3	115.7	97.7
Average	115.0	96.4	115.8	98.4
RSD	1.4	1.3	0.4	0.4
Pooled RSD of n=12	D1	1.1		
	D2	1.4		
Absolute mean assay difference	D1	0.8		
	D2	2.0		

Precision Cont…

Reproducibility

- Reproducibility with the same samples using different laboratories, analysts, days, reagent, and environmental conditions
- Analysis of an homogeneous sample in different laboratories, by different analysts, using the specified parameters
- Mainly applied for method Transfer

5. Range

- The interval between the upper and lower quantitation levels of analyte (including these levels) demonstrated by suitable precision, accuracy, and linearity

- Example 50 µg/mL – 150 µg/mL

Range Cont…

- The following minimum specified ranges should be considered
- Assay of drug substance or finished drug product
  - from 80% to 120% of the test concentration
- Content Uniformity and dissolution
  - minimum of 70% to 130% of the test concentration, unless otherwise justified

6. Limit of Detection / Quantitation

- Characteristic for low level impurity assays
  - LOD
    - The lowest amount of any analyte which can be detected but not necessarily quantitated
  - LOQ
    - The lowest amount of analyte that can be determined with acceptable precision and accuracy

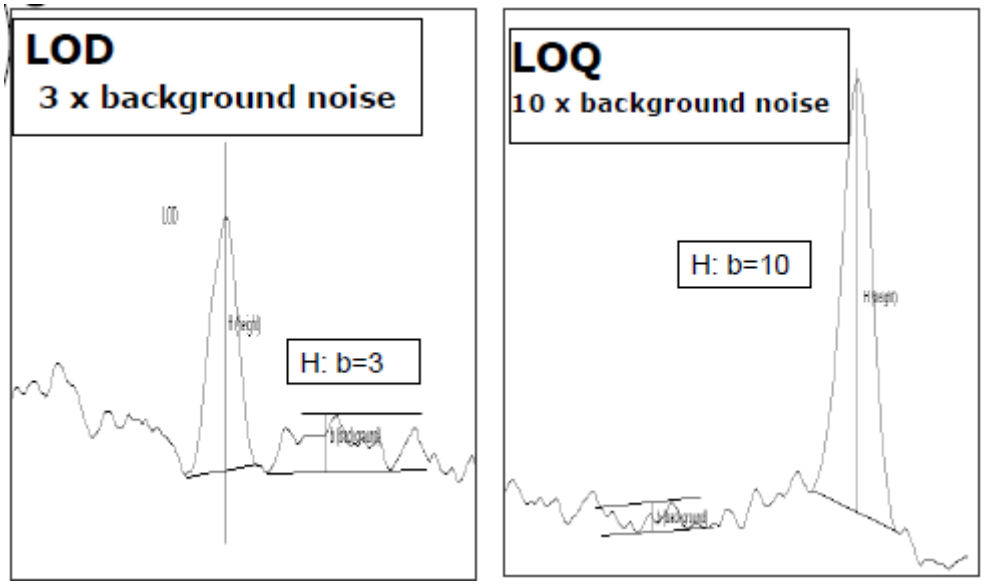
Limit of Detection

- Instrumental/Chromatographic
  - Can be made at a value at which the signal to noise ratio (S/N) is 3:1
- Non-instrumental Methods
  - Analysis of samples with known concentrations of analyte to establish the minimum concentration at which the analyte can be detected
  - Examples: TLC, color comparison

Limit of Quantitation

- The lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy.
  - Can be made at a value at which the signal to noise ratio (S/N) is 10:1

Detection Limit/ Quantitation Limit



7. System Suitability

- System suitability tests are an integral part of chromatographic methods.
- These tests are used to verify that the chromatographic system is adequate for the intended analysis.
- Prior to injecting a standard solution in creating the standard plot, it is essential to ensure that the system is performing adequately for its intended purpose.
- This function is fulfilled by the use of a solution of the system suitability.
- The tests are based on the concept that the equipment electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such.

System Suitability

- The following notes should be given due consideration when evaluating a system suitability:

- System suitability is a measure of the performance of a given system on a given day within a particular sample analysis set.
- The main objective of system suitability is to recognize whether or not system operation is adequate given such variability as chromatographic columns, column aging, mobile-phase variations, and variations in instrumentation.

System Suitability

- System suitability is part of method validation. Experience gained during method development will give insights to help determine the system suitability requirements of the final method.
  - ✓ Example, the hydrolysis of acetylsalicylic acid to salicylic acid in acidic media. Separation of this degradation peak from the analyte could be one criterion for the system suitability of an acetylsalicylic acid assay.
- A system suitability test should be performed in full each time a system is used for an assay.
- If the system is in continuous use for the same analysis over an extended period, system suitability should be reevaluated at appropriate intervals (bracketing).

System Suitability

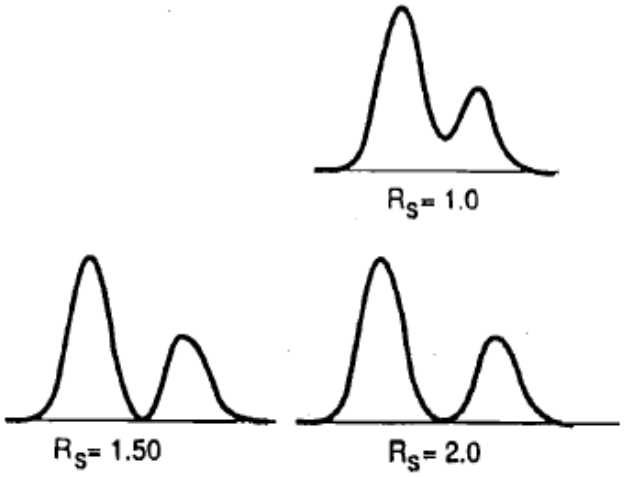
- Factors that may affect chromatographic behavior include the following
- Composition, ionic strength, temperature, and apparent pH of the mobile phase (external Factors)
- Flow rate, column dimensions, column temperature, and pressure (internal Factor)
- Stationary phase characteristics, including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area
- Reverse-phase and other surface modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading, etc.)

System Suitability

- System suitability should be based on criteria and parameters collected as a group that will be able to define the performance of the system.
- Some of the common parameters used includes:
  - precision of repetitive injections (usually five or six)
  - Resolution (*R),*
  - *Tailing factor (T)*
  - *Number of theoretical* plates (*N), and*
  - *Capacity factor (k*).

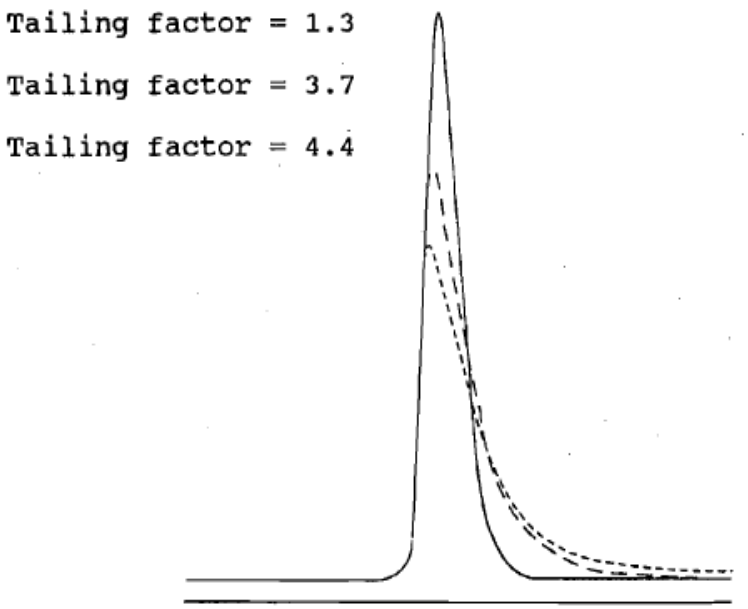
System suitability testing (SST)

1. Precision:
   - Assay: RSD ≤1% (API) or ≤ 2% (FPP), n ≥ 5
   - Impurities: in general, RSD ≤ 5% at the limit level, up to 10% or higher at LOQ, n ≥ 6
2. Resolution (R): >2



System suitability testing (SST)

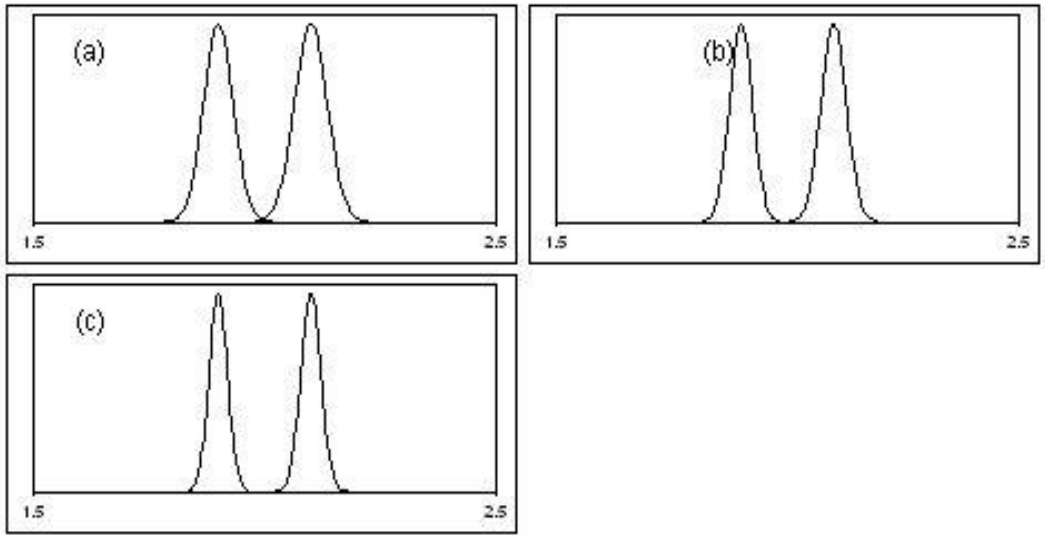
3. Tailing factor/peak asymmetry: (≤ 2)



System suitability testing (SST)

4. Number of theoretical plates (N): column efficiency ≥ 2000

Theoretical models of peaks obtained on columns packed with three different particle sizes: retention times 1.9 & 2.1 min on a 50mm column (a) particle size, d_p=5µm; theoretical plates, N=3500; resolution, R=1.48 (b) particle size, d_p=3µm; theoretical plates, N=5800; resolution, R=1.90 (c) particle size, d_p=1.9µm; theoretical plates, N=9200; resolution, R=2.40



System suitability testing (SST)

A SST should contain:

- For Assay:

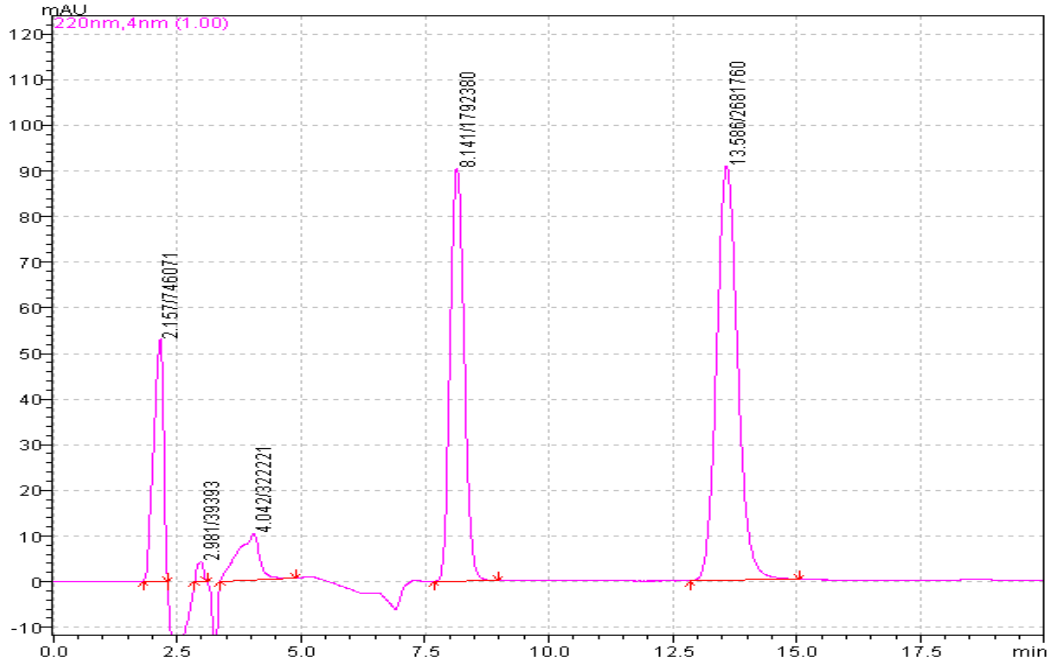
  Precision + one or more other parameter

- For impurity test:

  Resolution + precision + one or more other parameter

Example System suitability of an analytical method

The resolution (R) between the two analytical peak pairs is not less than 8.0; theoretical plate number is not less than 6000 for the two analytes and tailing factor should be between 0.9 and 1.5 for the two analytes.



7. Robustness

- The ability of the method to remain unaffected by small but deliberate variations in method parameters
- Provides an indication of the reliability of the method during normal usage.
- Evaluated by varying method parameters and determining the effect (if any) on the results of the method.
- If the results are susceptible to parameter variations, these parameters should be adequately controlled and a precautionary statement included in the method

Robustness Cont…

- Two approaches

A. Uni-variable (One factor at a time)

  - Systematically varying each parameter sequentially
  - Typically, each parameter may be varied by 5 – 10% above and below the value set in the method

B. Multi-variable

  - Use of the statistical design

Table: Internal variables (column temperature, mobile phase flow rate, detector wavelength and injection volume) of robustness testing of the proposed method.

Peak Characteristics	Column Temperature in ^{0}C (25)		Mobile Phase flow rate in mL per minute (1.0)		Detector Wavelength in (nm) (220)		Injection Volume in µL (20)	
	20	30	0.9	1.1	217	223	10	30
R	16.5	13.6	15.5	14.8	15.1	15.1	16.5	13.0
T*	1.3/1.1	1.2/1.1	1.3/1.1	1.2/1.1	1.2/1.1	1.3/1.1	1.2/1.1	1.2/1.1
N**	6421/6557	6618/6663	6646/7044	6421/6890	6282/6644	6326/6658	7458/8008	6050/6104

And ** for D1/D2 respectively

Table: External variables (pH, Ion pair concentration, organic con and triethylamine concentration of the mobile phase) of robustness testing of the proposed method.

Peak Characteristics	pH of the mobile phase (7.0)		Ion pair con. of the Mobile Phase (30)		Organic con. of the Mobile Phase (51)		Triethylamine con. of the Mobile Phase (5)	
	6.8	7.2	27 mM	33 mM	50 %	52 %	4 mL	6 mL
R	16.9	11.8	13.7	13.4	16.8	13.8	13.0	16.1
T*/**	1.3/1.1	1.2/1.1	1.2/1.1	1.2/1.1	1.2/1.1	1.2/1.1	1.2/1.1	1.2/1.1
N*/**	6315/7263	6568/6261	6511/6539	6471/6508	6663/7249	6390/6555	6447/6392	6479/7069

And ** for D1/D2 respectively

Revalidation

- Verification or revalidation should be performed when relevant, for example:
  - Changes in the synthesis of the drug substance
  - Changes in the composition of the drug product
  - Changes in the analytical procedure
  - when major pieces of equipment instruments change to another
  - when analytical methods are transferred.
- The verification or degree of revalidation depend on the nature of the change(s).

THANK YOU!