VALIDATION OF ANALYTICAL METHOD

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- **Introduction**

- Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

- Analytical methods need to be validated or revalidated:

  -Before their introduction into routine use;

  -Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and

  -Whenever the method is changed and the change is outside the original scope of the method.

Method Characteristics to Be Considered for Validation

- Specificity (Selectivity)
- Linearity
- Range
- Accuracy
- Precision
  - Repeatability
  - Intermediate Precision
  - Reproducibility (Ruggedness)
- Detection Limit
- Quantitation Limit
- Robustness
- System Suitability Testing
- Specificity and stability

Validation Characteristics

	Identification	Impurities		Assay
		Quantitative	limit	
Accuracy	-	+	-	+
Precision	-	+	-	+
Specificity	+	+	+	+
Detection Limit	-	-	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+
Robustness	+	+	+	+

Specificity

- Is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present (impurities, degradants, matrix…).

Identity testing

- To ensure the identity of an analyte.

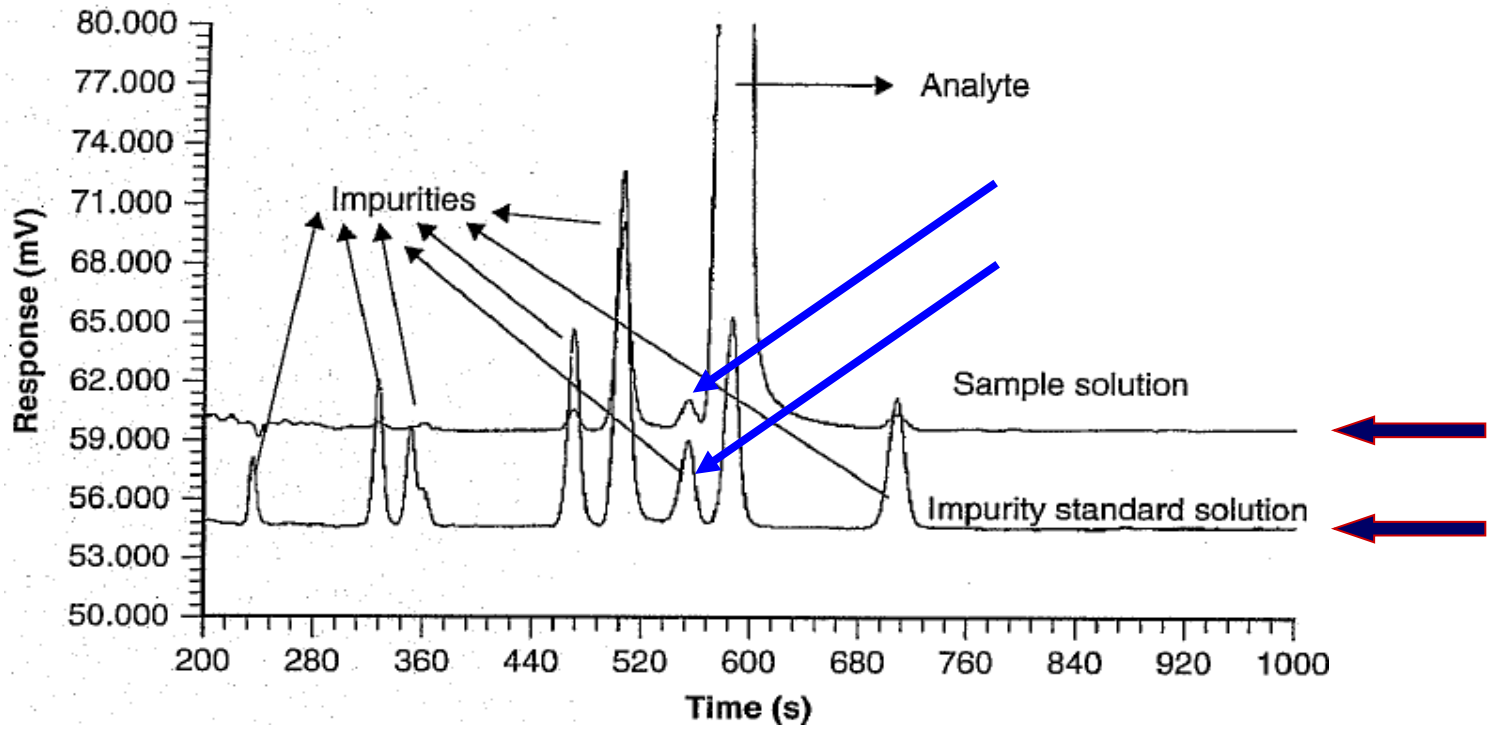
Purity testing

- To ensure accurate statement on the content of impurities of an analyte.

Assay

- To allow an accurate statement on the content of an analyte in a sample

- **Specificity:** **Overlay chromatogram of an impurity solution with a sample solution**



- **Linearity**

It is the ability (within a given range) to obtain test **results** which are directly **proportional to the concentration** (amount) of analyte in the sample.

- **If there is a linear relationship test results should be evaluated by appropriate statistical methods:**
  - **Correlation coefficient (r)**
  - **Y-intercept**
  - **Slope**

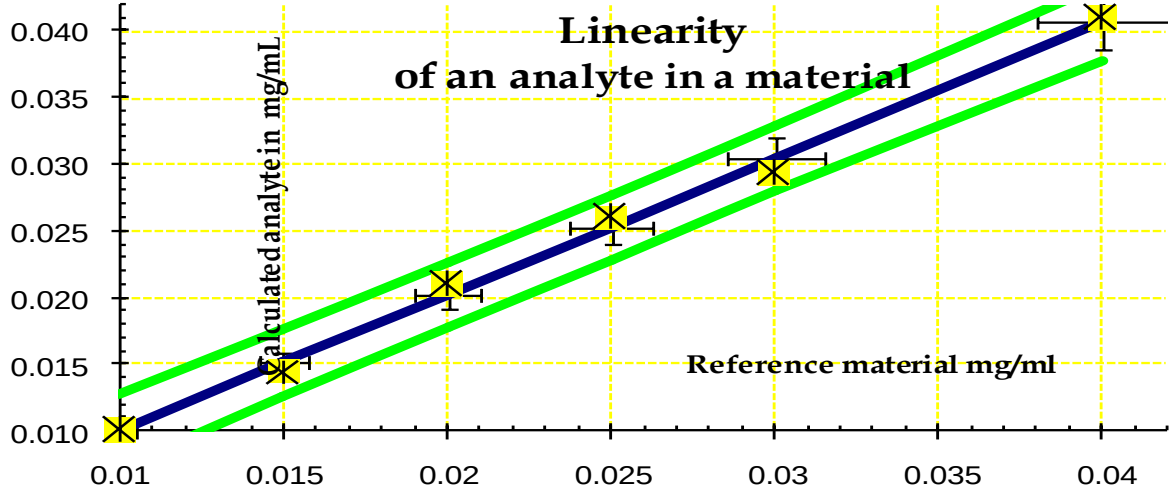


Table of values (x,y)

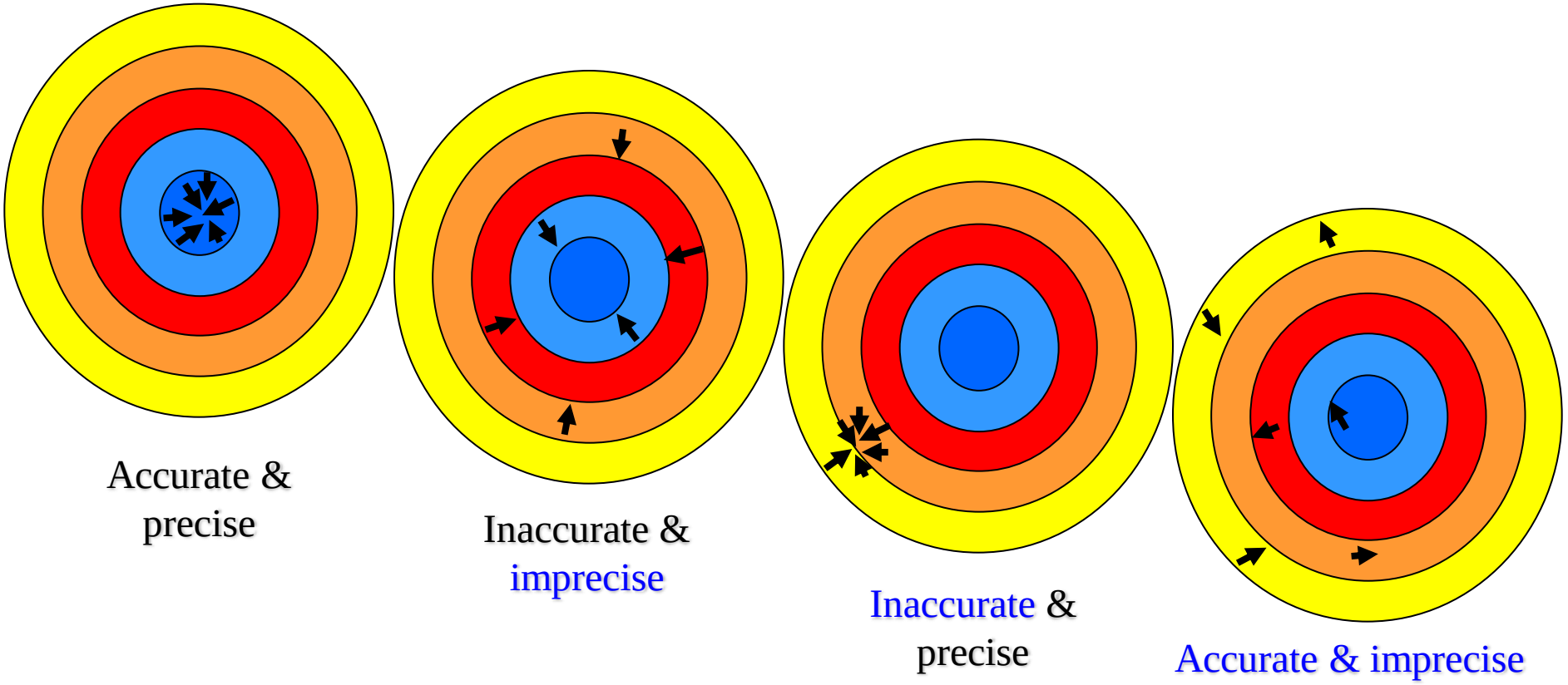
#	x Reference material mg/ml	y Calculated mg/ml
1	0.0100	0.0101
2	0.0150	0.0145
3	0.0200	0.0210
4	0.0250	0.0260
5	0.0300	0.0294
6	0.0400	0.0410

Linearity Statistics

- Intercept -0.0002
- Limit of Linearity and Range

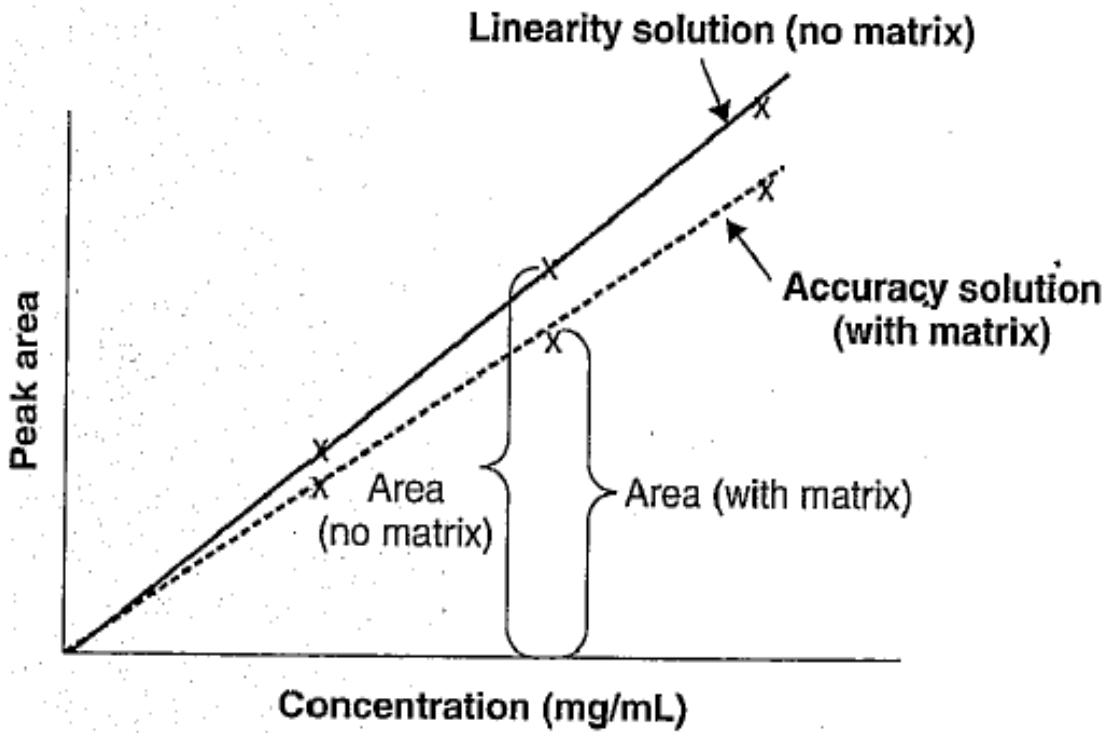
  0.010 – 0.040 mg/mL
- Slope 1.0237
- Correlation coefficient 0.9978

- **Accuracy and precision**



- **Accuracy**: Application of the method to synthetic mixtures of the drug product components to which known quantities of the analyte have been added.

- **Recovery reduced by ~10 – 15%**



- **Precision**
  - Expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample
  - Is usually expressed as the standard deviation (S) or coefficient of variation (RSD) of a series of measurements.
  - Precision may be considered at three levels:
    - **Repeatability** (intra-assay precision)
    - **Intermediate Precision** (variability within a laboratory)
    - **Reproducibility** (precision between laboratories)

Repeatability

- Six replicate sample preparation steps from a homogenously prepared tablet mixture (nominal value of API 150 mg)

Injection	Peak area	Assay
1	173865	98.06%
2	174926	98.66%
3	172933	97.54%
4	175011	98.72%
5	179557	101.30%
6	176425	99.52%
Mean	175453	98.96%
RSD	1.32%	1.32%

Intermediate precision

- Expresses within-laboratories variations (different days, different analysts, different equipment etc.)

Injection	Peak area analyst 1	Peak area analyst 2	Peak area analyst 3
1	173865	175656	177965
2	174926	175878	178556
3	172933	176004	177342
4	175011	176344	178011
5	179557	175332	179466
6	176425	174959	179688
Mean	175453	175695	178504
RSD	1.32%	0.28%	0.51%

Reproducibility

- Expresses the precision between laboratories:
- Collaborative studies, usually applied to standardisation of methodology
  - **Transfer of technology**
  - **Compendial methods**

Injection	Peak area analyst lab-1	Peak area analyst lab-2
1	175656	177965
2	175878	178556
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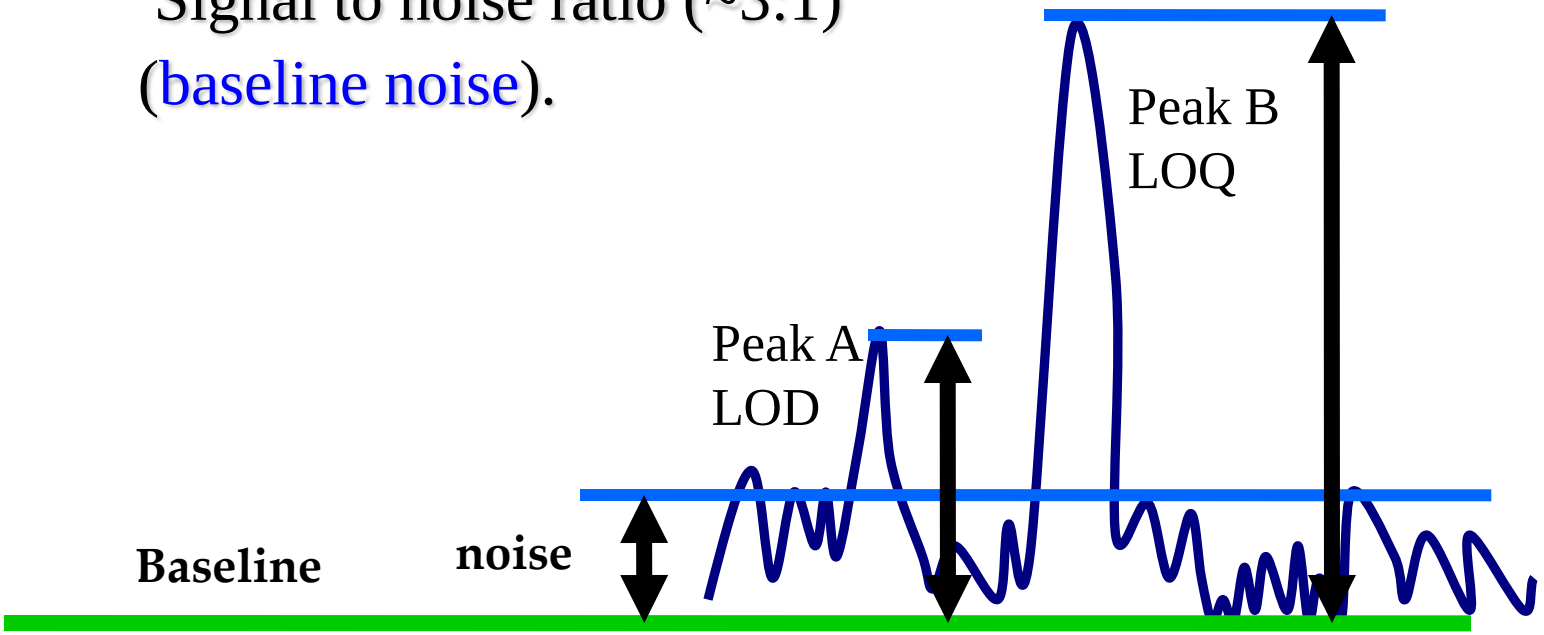
Mean	17569	178504
RSD	0.28%	0.51%

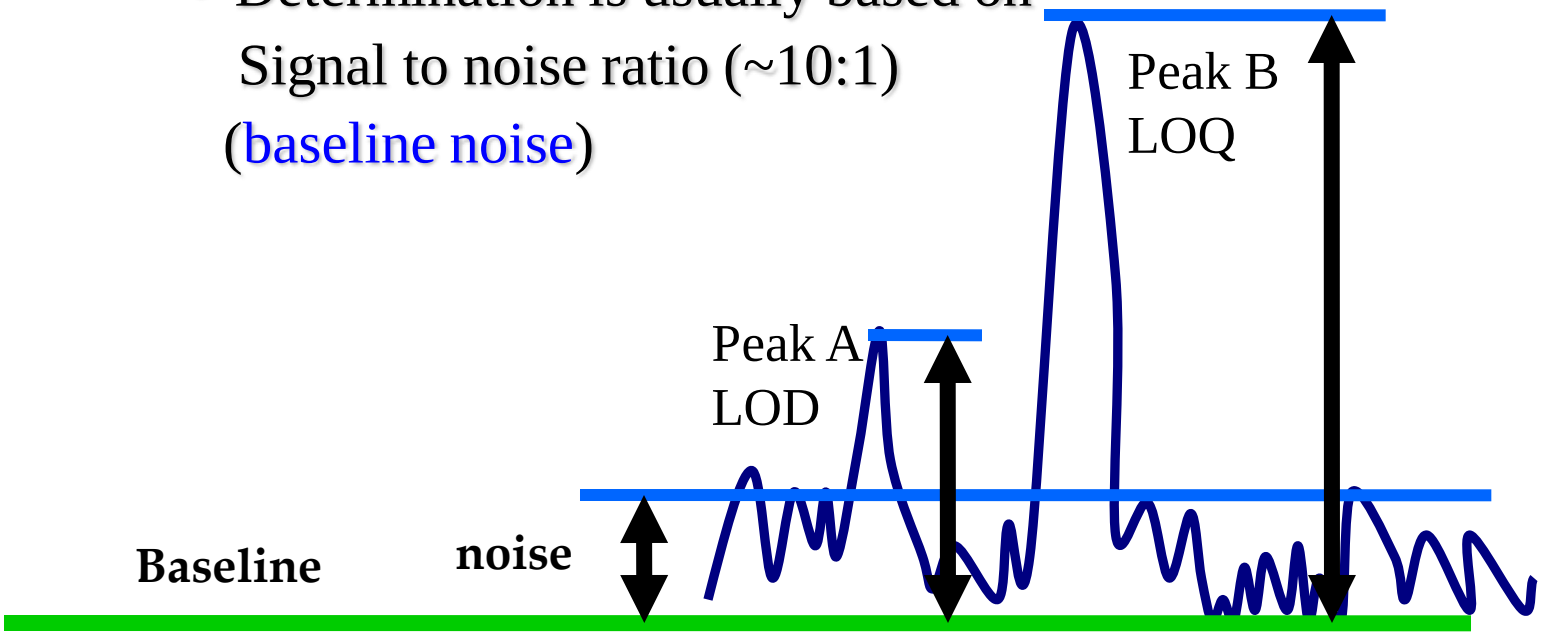
- **Range**
  - The range of an analytical procedure is the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity
  - Assay
    - 80 to 120% of test concentration.
  - Content uniformity
    - 70 to 130% of test concentration.
  - Dissolution
    - Q-20% to 120%.
  - Impurities
    - Reporting level – 120% of specification limit (with respect to test concentration of API).
  - Assay & Impurities
    - Reporting level to 120% of assay specification

- **Limit of Detection (LOD, DL):**
  - The LOD of an analytical procedure is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as an exact value.
  - Determination is usually based on Signal to noise ratio (~3:1) (baseline noise).



Limit of Quantitation (LOQ, QL)

- The LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy
  - The quantitation limit is used particularly for the determination of impurities and/or degradation products
  - Determination is usually based on Signal to noise ratio (~10:1) (baseline noise)



Specificity and stability:

- Stress stability testing to ensure the stability indicating potential of an analytical method
- Assure that the API can be assessed specifically in the presence of known and unknown (generated by stress) impurities.
- Assure that known impurities/degradants can be specifically assessed in the presence of further degradants.
- By peak purity assessment and (overlay of) chromatograms

- **Stress stability studies versus forced degradation studies**

Stress parameter	Forced degradation	Stress stability (5 – 15% decomposition)
Acid	0.2 ml **1N HCl** / 5 ml API-solution / **3h, 6h**, 12h, 24h…7d (RT & 60°C)	**pH** ± 2 (2 weeks)
Base	0.2 ml **1N NaOH** / 5 ml API-solution / **3h, 6h**, 12h, 24h…7d (RT & 60°C)	**pH** ± 10 (2 weeks)
H_2O_2 / Oxygen	0.2 ml **5%** or **35% H_2O_2** / 5 ml API-solution (RT, to 7d & 60°C, **3h**)	1 g/ml **oxygen** bubbled through (8 hours) 0.1 – 2% **H_2O_2** (24 hours)
Heat	**60°C** / 5 ml solution (**3h, 6h**…7d)	-
Heat	**105° C** / solid API (**1d** and 7d)	60°C (4 weeks)
UV or Light	365 nm or white fluorescent light / solid API (**1d** and 7d)	-
Humidity	-	50°C / 80% RH (4 weeks)

Robustness

- Robustness of an analytical procedure should show the reliability of an analysis with respect to deliberate variations in method parameters.
- The evaluation of robustness should be considered during the development phase.
- If measurements are susceptible to variations in analytical conditions the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

Influence of buffer pH and buffer concentration in mobile phase on retention times of API and impurities:

	API	Impurity A	Impurity B	Impurity C
As is	10.46	3.86	7.43	8.26
buffer pH 5.9	10.45	3.94	7.51	8.38
buffer pH 6.9	10.46	3.94	7.49	8.34
Buffer conc. 83%	**7.84**	**3.43**	**6.16**	**6.66**
Buffer conc. 87%	**15.26**	**4.77**	**9.61**	**11.18**

- **System suitability testing**
  - Based on the concept that equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such
  - Suitability parameters are established for each analytical procedure individually
    - Depend on the type of analytical procedure

Summary

- **Analytical procedures play a critical role in pharmaceutical equivalence and risk assessment/management**
  - Establishment of product-specific acceptance criteria
  - Assessment of stability of APIs
- **Validation of analytical procedures should demonstrate that they are suitable for their intended use**
- **Validation of analytical procedures deserves special attention during assessment of dossiers for prequalification**

THANK YOU