

# Validation of Analytical Methods



World Health  
Organization

# Outline

- Validation/Verification of the methods
  - Compendial methods Vs Non-compendial methods
- Summary of the analytical procedure and validation in QOS
- Method transfer

# Validation of Analytical Procedures

Testing a method to demonstrate it is suitable for its intended purpose and the results obtained are reliable, accurate and reproducible.

Provides confidence that the method will perform properly under intended conditions.

- Identification tests;
- Quantitative or limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. Including **assay, Content Uniformity, dissolution, content of preervatives.**

# Validation of Analytical Procedures

Characteristics	Type of analytical procedure			
	ID	Impurities		Assay
		limit	quantitative	
Specificity	+	+	+	+
Accuracy			+	+
Precision				
Repeatability			+	+
Intermediate precision			+	+
Linearity/Range			+	+
Limit of detection		+	+	
Limit of quantitation			+	



# Specificity

- Specificity is the ability to measure specifically the analyte in the presence of other components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.
- Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

# Specificity

- Blank solution - to show no interference
- Placebo - to demonstrate the lack of interference from excipients
- Spiked samples - to show that all known related substances are resolved from each other
- Stressed sample of about 10 to 20% degradation is used to demonstrate the resolution between degradants and the analyte of interest
  - Check peak purity of drug substance by photodiode array detector (PDA)
- Representative chromatograms should be provided

# Accuracy

- Expresses the closeness of test results obtained by that procedure to the true value= trueness
  - the value accepted as a conventional true value, or
  - an accepted reference value and the value found
- accuracy should be established across the specified range of the analytical procedure



# Accuracy

## Assay

**API:** against an RS of known purity, or via an alternate method of known accuracy; analysis in triplicate.

**FPP:** placebo/drug product spiked with known quantity of API, in triplicate at each level (80, 100 and 120% of label claim) is recommended.

Report recovery (mean result and RSD): 98.0-102.0%

ICH Q2 states: accuracy may be inferred once precision, linearity and specificity have been established. (Demonstration preferred).



# Accuracy

## Impurities:

API/FPP spiked with known amounts of impurities

## Recommendations:

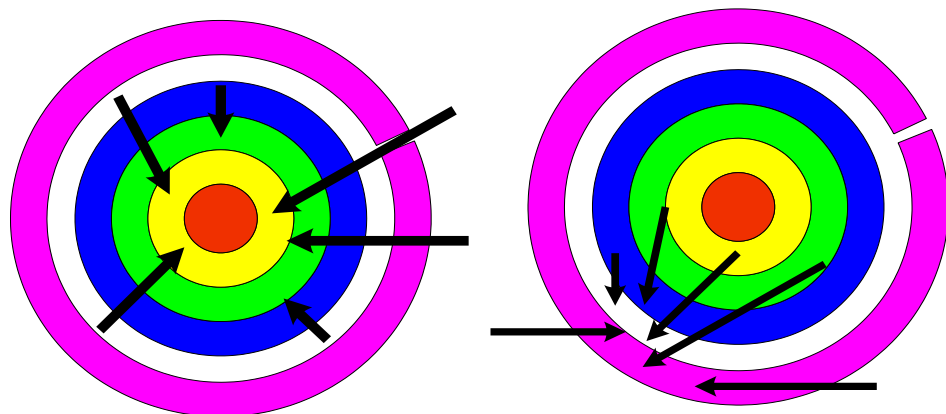
Across the range of LOQ-150% of the target concentration (shelf life limit), 3-5 concentrations, in triplicate each. (LOQ, 50%, 100%, 150%)

Per cent recovery: in general, within 80-120%, depends on the level of limit

# Precision

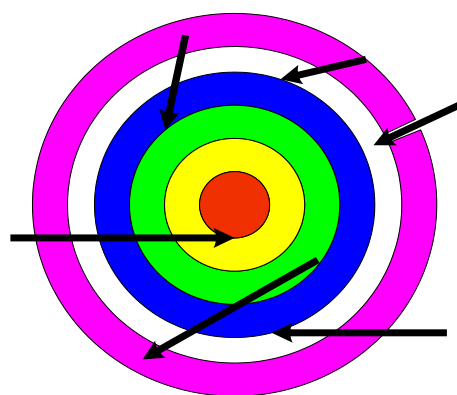
- Precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of test results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.(degree of agreement among individual test results).
- It should be measured by the scatter of individual results from the mean and expressed as the relative standard deviation (RSD). May be considered at three levels
  - Repeatability
  - intermediate precision (ruggedness)
  - reproducibility.

# Accuracy & Precision

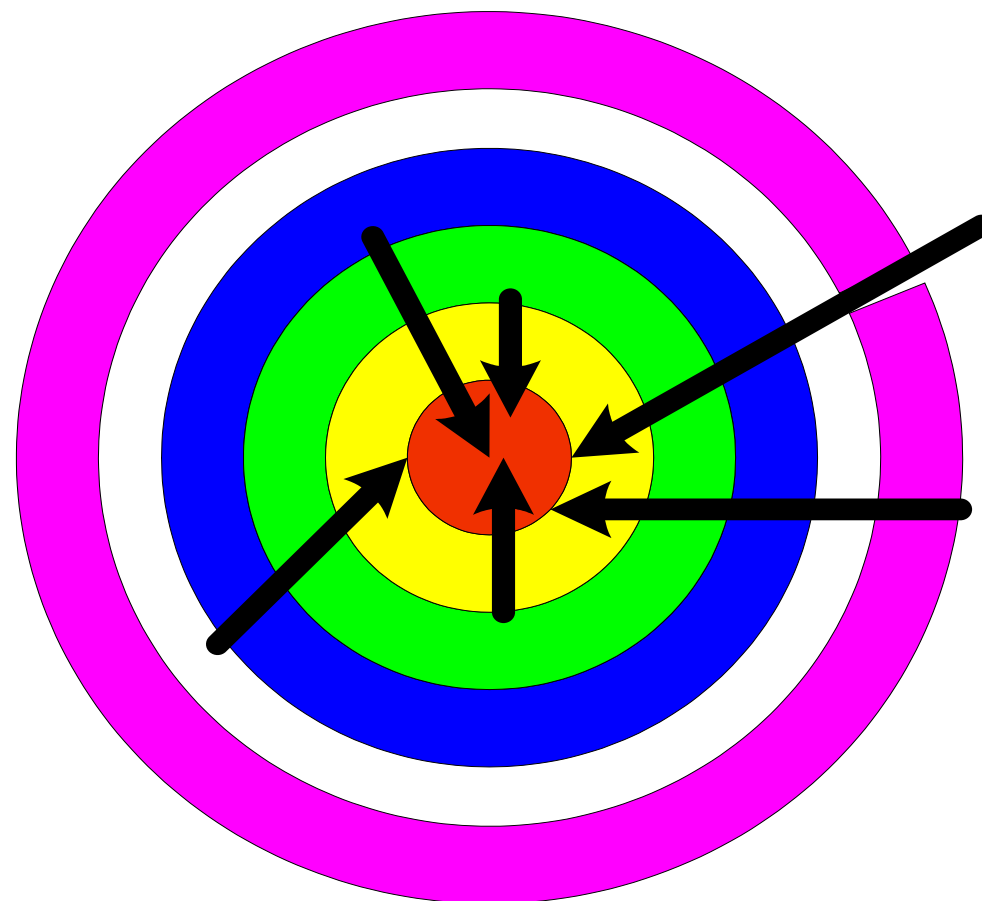


Accurate but imprecise

Inaccurate but precise



Inaccurate & imprecise



Accurate and precise

# Precision

- Repeatability (method precision)
  - Multiple measurements of a sample by the same analyst
  - A minimum of 6 determinations at the test concentration (6 times of a single batch), or
  - 3 levels (80%, 100%, 120%) , 3 repetitions each

Recommendation:

- For Assay:  $RSD \leq 2.0\%$
- For individual impurity above 0.05%, in general,  $RSD \leq 10\%$

# Precision

- Intermediate precision
  - Test a sample on multiple days, analysts, equipments
  - RSD should be the same requirement as method precision
- Reproducibility (inter-laboratory trial)
  - Not requested in the submission
  - Need to be considered for method transfer

# Precision

	Repeatability Condition	Intermediate Precision Condition	Reproducibility Condition
Laboratory	Same	Same	Different
Operator	Same	Different	Different
Apparatus	Same	Same <sup>a</sup>	Different
Time between Tests	Short <sup>b</sup>	Multiple Days	Not Specified

<sup>a</sup> This situation can be different instruments meeting the same design requirement.

<sup>b</sup> Standard test method dependent, typically does not exceed one day

Matrix design can be used for intermediate precision and reproducibility



# Linearity / Range

- The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
- The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
- Minimum 5 concentrations

# Linearity / Range

- Assay : 80-120% of the test concentration
- Content Uniformity: 70-130% of the test concentration
- Dissolution:  $\pm 20\%$  of limits; eg if limits cover from 20% to 90% l.c. (controlled release), linearity should cover 0-110% of l.c.
- Impurities: LOQ to 120% of shelf life limit
- Assay/Purity by a single method: LOQ of the impurities to 120% of assay limit



# Linearity / Range

Correlation coefficient (r)

Assay:  $r \geq 0.999$

Impurities:  $r \geq 0.99$

y-Intercept and slope should be indicated together with plot of the data

# LOD/LOQ

- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
- The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

# LOD/LOQ

- Visual evaluation (non-instrumental method)
- signal to noise ratio: LOD 3:1 , LOQ 10:1
- standard deviation of the response and the slope of the calibration curve at levels approximating the LOD /LOQ

$$DL = \frac{3.3\sigma}{S}$$

$$QL = \frac{10\sigma}{S}$$

$\sigma$  = the standard deviation of the response

S = the slope of the calibration curve

should be validated by analysis of samples at the limits.

# LOD/LOQ

- LOD: below the reporting threshold
- LOQ: at or below the specified limit

Not required for assay/dissolution methods.

- Applicant should provide
  - the method of determination
  - the limits,
  - chromatograms

# Robustness

- The method's capability to remain unaffected by small but deliberate variations in method parameters (for HPLC)
  - Influence of variations of pH in a mobile phase
  - Influence of variations in mobile phase composition
  - Different columns (different lots and/or suppliers)
  - Temperature
  - Flow rate
- Establish the System suitability parameters
- If robustness indicates a limitation, this must be clearly stated in the method

# Note

- The recommended acceptance criteria for each validation characteristics in this presentation are mainly for validation of HPLC method. It is based on normal process and experiences in the review of prequalification submissions.
- Other approach for validation characteristic may be applicable and acceptable. It is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose.

# Revalidation

Revalidation may be necessary

- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;
- Changes in the analytical procedure.

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# Compendial versus in-house standards





# Validation, verification, equivalency

- **Validation:** full validation required for in-house methods, generally specificity, linearity, accuracy, repeatability, intermediate precision, plus for purity: LOD/LOQ.
- **Verification:** required for most compendial methods
- **Equivalency:** required for an in-house method, when compendial standard is claimed

# When compendial methods are adopted

Verification data is required.

- The compendial methods as published are typically validated based on an API or an FPP originating from a specific manufacturer.
- Different sources of the same API or FPP may contain impurities and/or degradation products that were not considered during the development of the monograph.
- The FPP may also contain excipients not present in the FPP(s) for which the compendial method was developed.

**Therefore, the suitability of compendial methods should be verified under actual conditions of use for the specified API or FPP matrix.**

# When compendial methods are adopted

## ● API

Assay: No validation generally required. Exception: specificity for any specified impurities not in the monograph.

Purity:

- Full validation for specified impurities that are not included in the monograph (specificity, linearity, accuracy, repeatability, intermediate precision, LOD/LOQ).

# When compendial methods are adopted

## ● FPP

- Assay: Specificity, accuracy and precision (repeatability). Eg. to rule out excipient interference.
- Purity:
  - Specificity, accuracy and precision (repeatability) for known imps.
  - Full validation for specified impurities that are not included in the monograph (specificity, linearity, accuracy, repeatability, intermediate precision, LOD/LOQ)

# Compendial standard claimed, but in-house method used

- Full validation is required for an in-house purity/assay method.
- **Equivalency** must be demonstrated compared to the compendial method
  - Assay: same samples using the two methods;
  - Related substances: same samples **spiking** with **specified** impurities (at the limits) using the two methods
- If equivalency is not demonstrated, it can not be claimed as compendial standard

# Equivalency

Equivalency is essentially two things:

- Ensure the in-house method is not inferior (in which case they should adopt the compendial method)
- Ensure the compendial test can be met, if applied.
- Two samples are sufficient.

Note that a compendial method becomes an in-house method when adjustments are made outside the allowable adjustments (Int.Ph, USP, EP general chapter Chromatography)

# Reporting Validation

- **Specificity:** chart of Rf or RRT values and discussion, if necessary.
- **Accuracy:** Conc. \_\_\_, recovery \_\_\_, RSD = \_\_\_; n = \_\_\_
- **Precision:** Conc. = \_\_\_, n = \_\_\_, RSD = \_\_\_
- **Linearity/Range:** range = \_\_\_ - \_\_\_; corr. coeff. = \_\_\_; slope = \_\_\_
- **Sensitivity:** LOD = \_\_\_; LOQ = \_\_\_;
- QOS 3.2 R.2 (example)

# Transfer of analytical procedures





# Transfer of analytical procedures

- **Method transfer** is the documented process that qualifies a laboratory (the receiving unit) to use an analytical test procedure that originated in another laboratory (the transferring unit).
- To ensure the receiving unit has the procedural knowledge and ability to perform the transferred analytical procedure as intended.

# Transfer of analytical procedures

USP <1224>:

- Several approaches to demonstrate:
  - Comparative testing on homogeneous lots of the target material, e.g. same lots of standard production batches or samples spiking of known impurities
  - Covalidation between laboratories, i.e. including the receiving unit in an interlaboratory covalidation to obtain data for the assessment of reproducibility.
- A preapproved transfer or validation protocol that stipulates the details of the procedure should be used.

# Transfer of analytical procedures

## USP <1224>

- The potential components of a transfer protocol
  - objective, scope, responsibilities,
  - instruments and analytical procedure
  - materials: samples, reference standards
  - experimental design: specific characteristics to be evaluated, analysis to be used to evaluate the outcomes
  - acceptance criteria
  - evaluation of the outcomes: report forms, handling deviations/failure results

# Transfer of analytical procedures

## USP <1224>

- The possibility of waiver
  - Compendial method—verification
  - Same or similar to a procedure already in use at the receiving unit
  - Similar product to the existing one analysed at the receiving unit: comparable composition, similar concentration of API.
  - The personnel in charge of the analysis/development/validation are moved to the receiving unit

# Transfer of analytical procedures

PQP practice

**Method transfer** that should include specificity and reproducibility. Reproducibility should be demonstrated by providing results of analysis of a minimum of 2 batches at each test site.

- Impurities: comparable results for samples spiked with the known impurities at concentrations which are close to their specification limits.

# Transfer of analytical procedures

PQP practice cont.

- When analytical methods have been developed in a GMP compliant facility and the original site included intermediate precision, method transfer report may not be required to be submitted, but they should be available.

# In conclusion

- To evaluate the clarity and completeness of the description of the analytical methods
- To confirm the sameness to compendial methods if compendial standard is claimed
- To review the verification/validation/equivalent data and confirm the suitability for the intended use
- Review QOS 3.2.R.2 and confirm with the submitted dossier

# Thank you !

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