



ICH Q2(R2): VALIDATION OF ANALYTICAL PROCEDURES ICH Q14: ANALYTICAL PROCEDURE DEVELOPMENT

Training Module 6: Multivariate Analytical Procedure

PART A - Introduction

- Types of Analytical Procedures
- Multivariate Analytical Procedures
- Near-Infrared Spectroscopy: Considerations
- Multivariate Elements in ICH Q14
- Multivariate Elements in ICH Q2 (R2)

PART B - Examples

- Example 1: Raman Spectroscopy for Identity Testing
- Example 2: Near-Infrared Spectroscopy for Assay
- Example 3: Raman Spectroscopy for Glucose Testing

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International Council for Harmonisation of Technical Requirements
for Pharmaceuticals for Human Use

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The materials presented in this ICH Q2(R2) / Q14 module are example approaches relating to selected aspects of analytical procedure development, validation and lifecycle. The approaches presented have been constructed to illustrate potential applications of the principles contained within the ICH Q2(R2) / Q14 guidelines and are not considered to be exhaustive. The examples are not intended to be mandatory, and alternative approaches (fulfilling the intent of the guidelines) may also be acceptable.

In some cases, additional elucidation of specific approaches is provided to aid in general understanding of a concept. This is not intended to be a promotion of the elucidated approach, nor indicate a preference for a specific approach.

Provision of acceptance criteria has been deliberately limited within this training material.

In practice, scientific rigor must be applied on a case-by-case basis when determining an appropriate approach or criterion.

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Part A - Introduction

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Types of Analytical Procedures

Analytical procedures can be classified in many ways. One of them is the complexity of sample preparation. Let's consider two cases:

1. Traditional chromatographic (e.g., LC-UV) analytical procedures.
 - Separation of sample components allows using single wavelength detection systems and simplifies data processing. This enables a univariate analysis (e.g., peak area) to be carried out, taking the chromatographic peaks into account separately.
 - This is done at the cost of complex sample preparation, often the most time-consuming part of analysis, resulting in longer analysis times.
2. Class of analytical procedures (e.g., Near-Infrared (NIR) spectroscopy) that does not require complex sample preparation.
 - These are usually based on spectroscopic principles and detection systems able to register multiple channels at the same time. In many cases, the sample can be analysed without any preparation.
 - Numerous spectroscopic responses (signals) are simultaneously interpreted using multivariate analysis. Spectroscopic approaches provide information based on the entire spectrum being analysed.

Multivariate Analytical Procedures

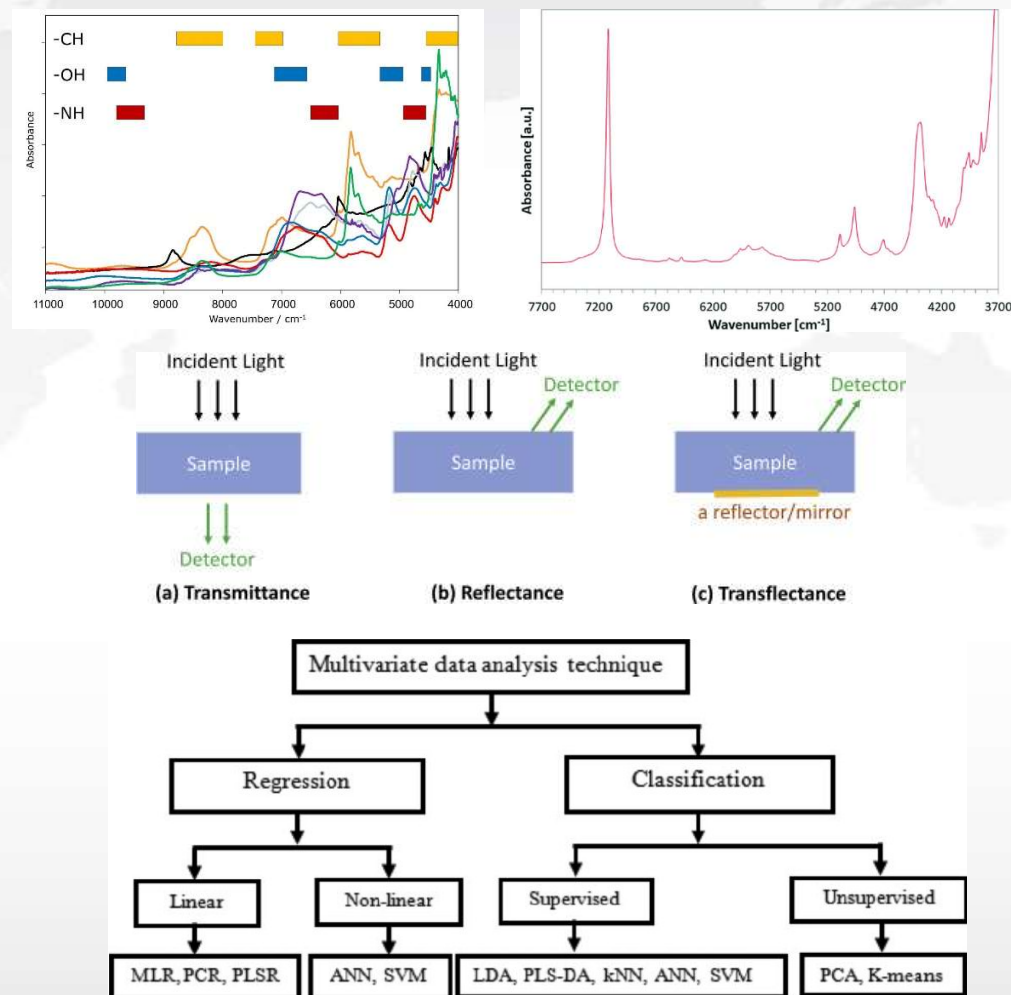
- No or little sample preparation combined with very short time of data acquisition and analysis (down to seconds).
- Collecting process data in real time allows process monitoring and process control.
- This is particularly important for continuous manufacturing due to:
 - Ability of controlling individual steps and real-time feedback and feedforward control.
 - Rejection of non-conforming in-process material.
- Higher capability to detect outliers caused by:
 - Changes in the process or properties of raw materials.
 - Changes in the interface (e.g., broken fibers).
- Capability to be deployed for multiple analytical purposes (see slide 8: NIR spectroscopy considerations).

Multivariate Analytical Procedures

- Complex data analysis procedures – multivariate model procedures (see slide 8: NIR spectroscopy considerations)
- Requires high level of expertise from procedure developers. Since multivariate models include information about sample matrix and optical properties of individual components, their change might reduce the quality of predicted results. Therefore, a periodical model performance check (and update, as needed) is necessary.
- In addition to being able to analyze complex data, these procedures (in combination with data pretreatment) can minimise undesirable effects caused by solid state of samples and superimposed signals from multiple components present in unprocessed samples.
- Generally, multivariate analytical procedures require more effort for development and maintenance; however, carrying out such a procedure routinely is much simpler and faster.

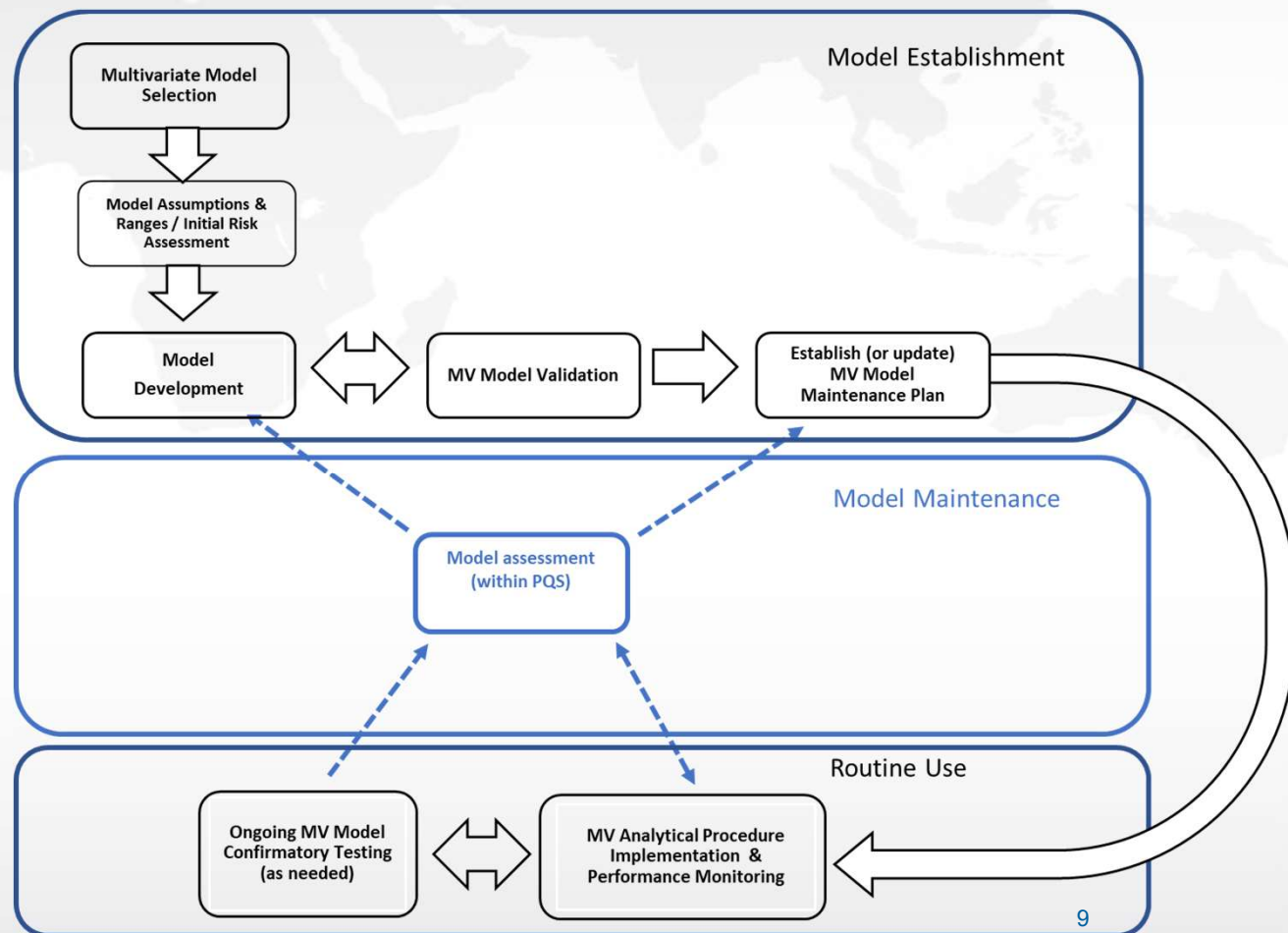
Near-Infrared Spectroscopy (NIR): Considerations

- **Chemical sensitivity:** chemical information on all components in a sample (e.g., drug substance(s) and excipients) are captured in the NIR spectra.
- **Physical sensitivity:** physical attributes that influence the path of the light (e.g., sample density and particle size) are captured in the NIR spectra.
- Because of the large amount of variable data captured in an NIR spectrum, reference analytical procedures and multivariate data analysis tools must be used in NIR analytical procedures.



Multivariate Elements in ICH Q14

- Development of multivariate analytical procedures.
- Documentation of multivariate analytical procedures.
- Example of multivariate model lifecycle components (Annex)



Multivariate Elements in ICH Q2(R2)

The below table is a copy from ICH Q2 (R2) Annex 2

Technique	NIR analytical procedure for core tablet assay
Performance characteristic	Validation testing methodology
Specificity/ Selectivity	<u>Absence of interference:</u> Comparison of drug substance spectrum and the loading plots of the model Rejection of outliers (e.g., excipient, analogues) not covered by the multivariate procedure
Precision	<u>Repeatability:</u> Repeated analysis with removal of sample from the holder between measurements
Accuracy	<u>Comparison with an orthogonal procedure:</u> Demonstration across the range through comparison of the predicted and reference values using an appropriate number of determinations and concentration levels (e.g., 5 concentrations, 3 replicates) Accuracy is typically reported as the standard error of prediction (SEP or RMSEP)
Reportable Range	<u>Response:</u> Demonstration of the relationship between predicted and reference values <u>Error (accuracy) across the range:</u> Information on how the analytical procedure error (accuracy) changes across the calibration range, e.g., by plotting the residuals of the model prediction versus the actual data
Robustness and other considerations	<u>Deliberate variation of parameters, e.g.,</u> Chemical and physical factors that can impact NIR spectrum and model prediction should be represented in data sets. Examples include various sources of drug substance and excipients, water content, tablet hardness, and orientation in the holder

Training Module 6: Multivariate Analytical Procedure

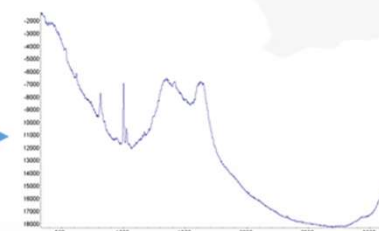
Part B - Examples

- Example 1: Raman Spectroscopy for Identity Testing
- Example 2: Near-Infrared Spectroscopy for Assay
- Example 3: Raman Spectroscopy for Glucose Testing

Example 1: Raman Spectroscopy for Identity Testing

- Intended purpose (element of the Analytical Target Profile (ATP)):
 - Identity test for biological drug product (liquid solution in vial)
- Technology selection:
 - Analytical technology requirements:
 - Simple and fast analysis (seconds to minutes)
 - Preferably non-destructive
 - No need for sample preparation

Simple analysis through DP vials



Raman spectrum

Outcome: Raman Spectroscopy with discriminant analysis

Example 1: Raman Spectroscopy for Identity Testing

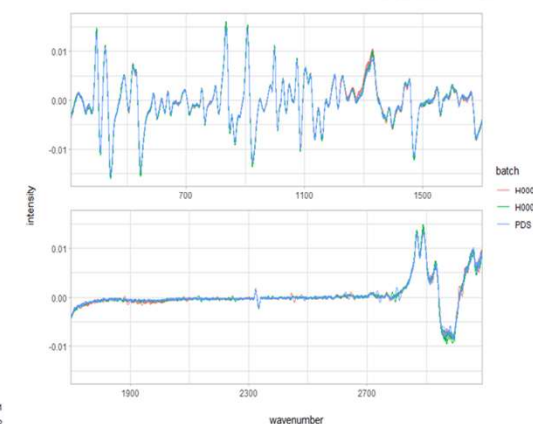
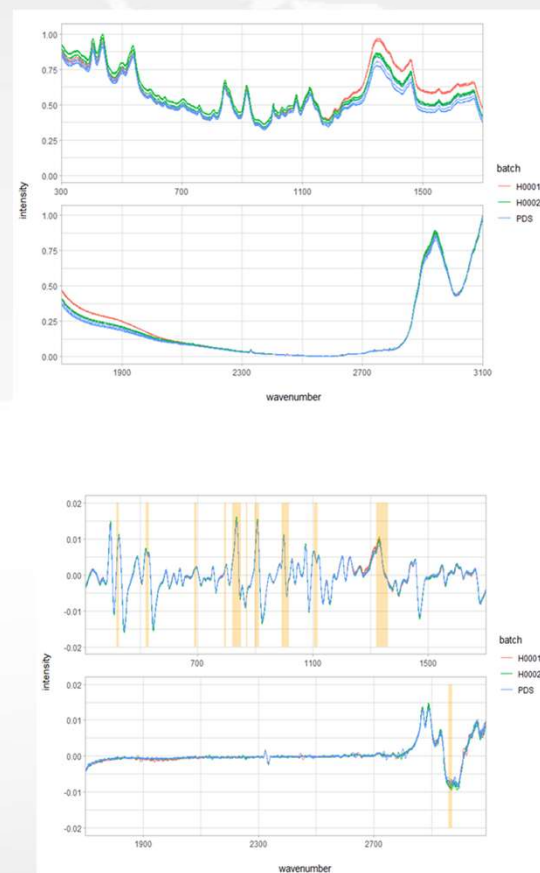
Model development – Calibration set design

- For each product specific multivariate identity model, 30 independent spectra are collected and used for model development, 2/3 of those spectra represent the calibration set:
 - Three drug product batches (3)
 - One sample per batch, analysed five times (3 x 5)
 - Two instruments, each $(3 \times 5) \times 2 = 30$ spectra
- Model development was informed by a risk assessment of variables that can affect the performance of the Raman procedure.
- Raw material variability as well as batch-to-batch variability were considered and included in the design of the spectral library.
- See slide 14 for graphical representation

Example 1: Raman Spectroscopy for Identity Testing

Model development

- Normalise spectra to reduce the impact of physical sensitivity (e.g., a Savitzky-Golay first derivative was employed to remove baseline effects).
- Select specific regions of normalised spectra to reduce impact of chemical sensitivity (e.g., to optimise the distinction of the product of interest from all other products processed in the same manufacturing area).
- Choose the multivariate approach (e.g., discriminant analysis)



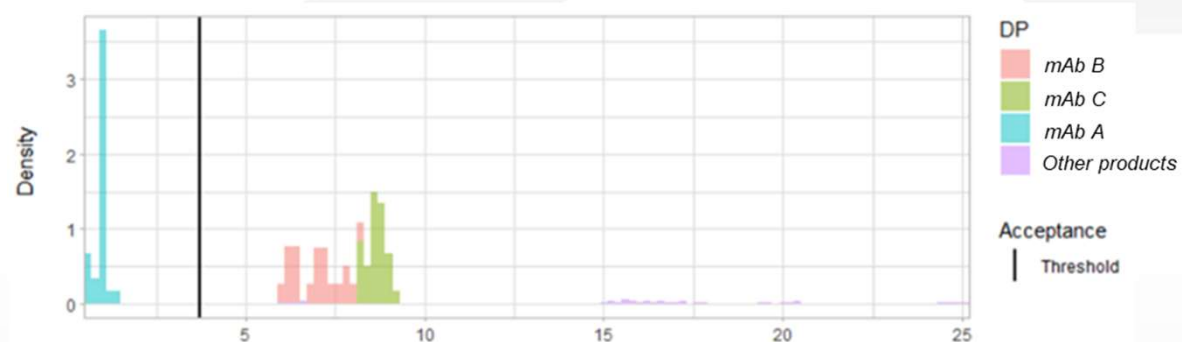
Example 1: Raman Spectroscopy for Identity Testing

Model development – Discriminant analysis

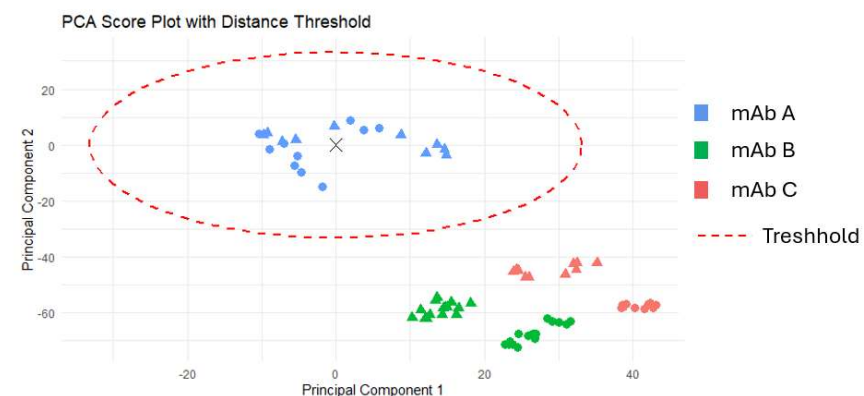
- The discriminant analysis reduces the dimensionality of the pre-processed data.
- The collected spectral data is pre-processed and projected into the Principal Components Analysis (PCA) space of the product-specific identity model
- A Mahalanobis distance is then calculated between the unknown projected point and the centroid of the product-specific identity model.
- Product identity is confirmed if the Mahalanobis distance is below the distance threshold of the product specific model.
- Vice versa, if the Mahalanobis distance is higher than the threshold, confirmation that the product identity test has failed, see next slide for graphical representation.

Example 1: Raman Spectroscopy for Identity Testing

Model development – Discriminant analysis (continued)



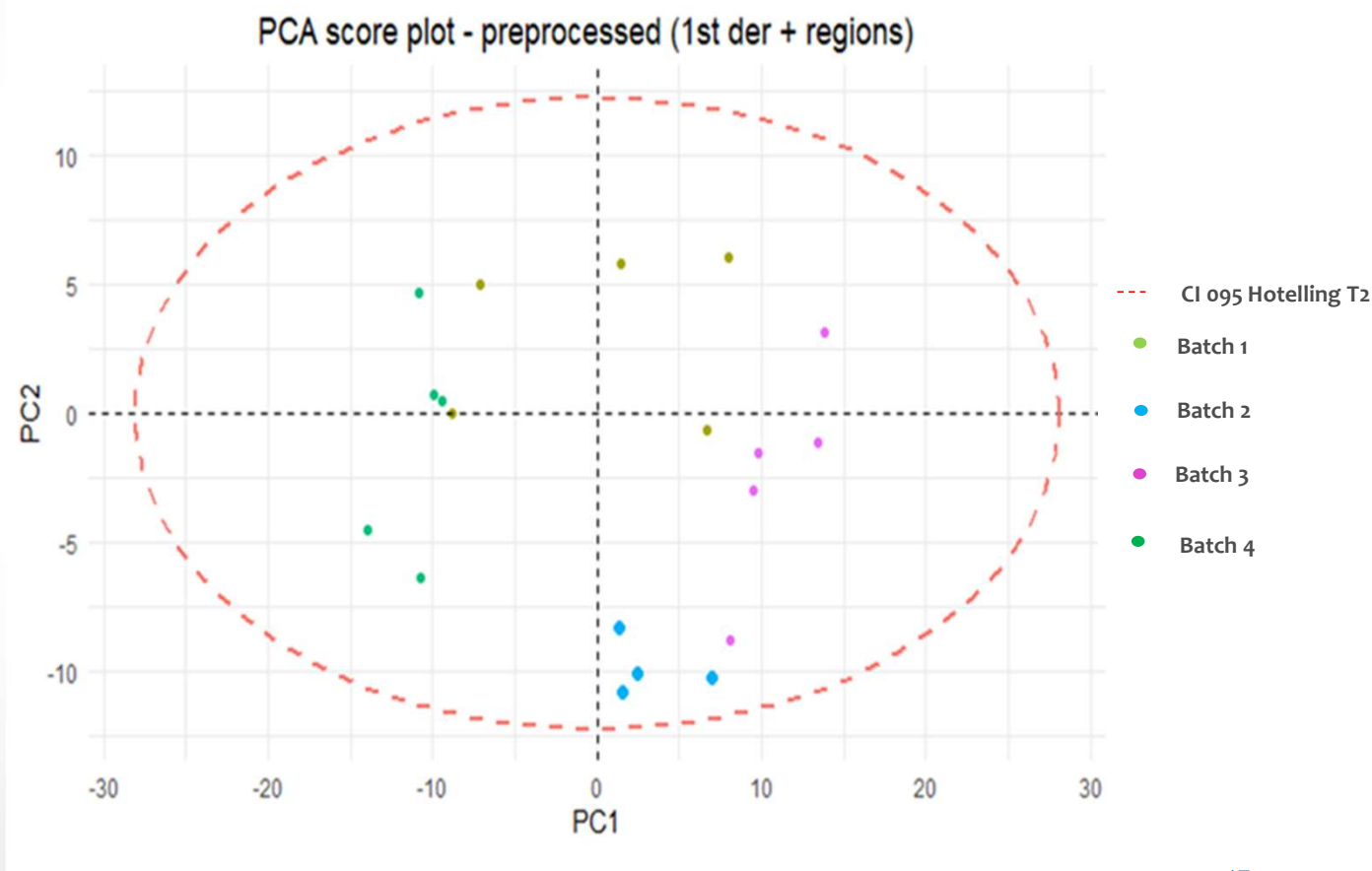
Mahalanobis distance considering all principal components



Euclidian distance based on subset of principal components

Example 1: Raman Spectroscopy for Identity Testing

- Exemplary PCA Score Plot of multiple Raman spectra from the same vial analysed on one instrument of the respective drug product batches for mAb A.
- Data points within the Hotelling T2 ellipse on PCA score plot indicate close similarity.
- Comparison of batches and replicates confirms low level of variability.



Example 1: Raman Spectroscopy for Identity Testing

Internal Testing

	Calibration Set	Internal Test Set
Product of interest	20 spectra (10/instrument), representing 2/3 of the data set in line with common practice for multivariate model building.	10 spectra (5/instrument) used to optimise Mahalanobis distance threshold and verify correct assignment of wavenumber regions, and for data quality check.
All other drug products manufactured in the same area	-	Several hundreds of spectra comprised of at least 10 spectra/drug product/ formulation (5/instrument) used to optimise Mahalanobis distance threshold and selection of product-specific wavenumber regions, and for data quality check.

Example 1: Raman Spectroscopy for Identity Testing

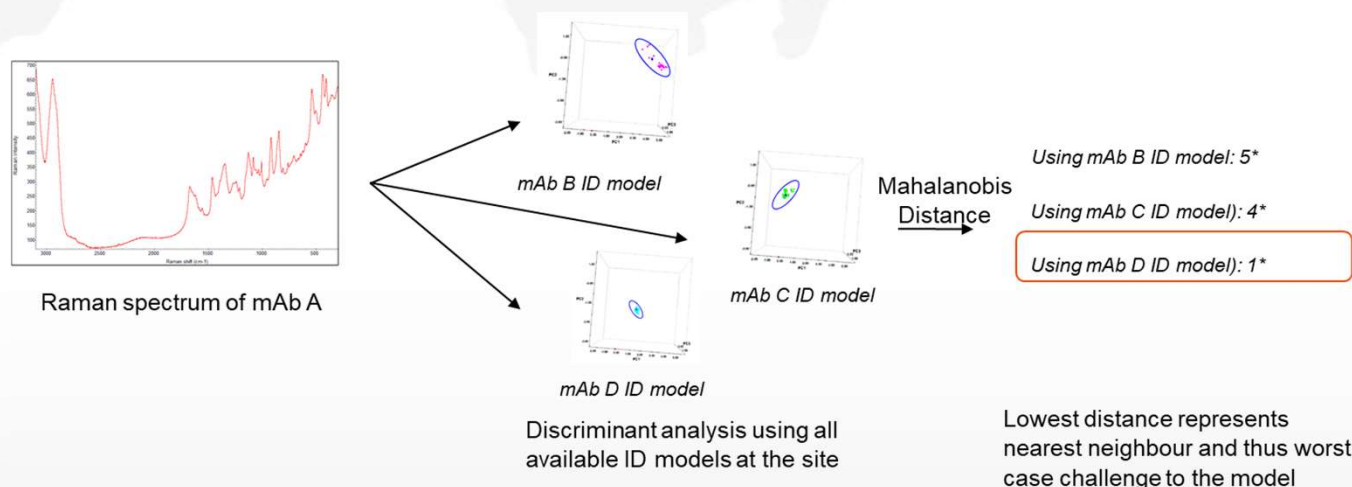
Model validation typically includes:

- Verifying the proper selection of wavenumber regions specific for the product of interest.
- Confirming that there are no conflicts among products of the spectral library.
- Confirming that Mahalanobis distance threshold is appropriately set.
- The validation set is an independent subset of the calibration data and includes Raman spectra of the product of interest (for positive control) as well as all other drug products (negative controls).

Example 1: Raman Spectroscopy for Identity Testing

Model validation

- All spectra of the product of interest were correctly identified during validation (true positive). All other drug products manufactured in the same manufacturing area were identified as true negatives (failed for the product of interest identity model)
- Nearest neighbour approach for negative controls



* Arbitrary numbers for illustration purposes only

Example 2: NIR Spectroscopy for Assay

This example is described in module 7 NIR in more detail.

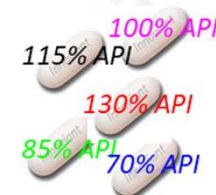
- Intended purpose (element of the ATP):
 - A market release analytical procedure for the assay for continuously manufactured tablets.
- Technology selection:
 - Analytical technology requirements:
 - Simple and fast analysis (seconds to minutes)
 - Non-destructive and no need for sample preparation
 - At-/On-/In-line analysis

Outcome: At-line NIR spectroscopy

Example 2: NIR Spectroscopy for Assay

Model development – Calibration set design

- Core tablets with different drug substance concentrations were made at the continuous manufacturing line at the development site. The variation in final drug substance concentrations was obtained by changing the excipient compositions.
- At the 100% level, core tablets with 3 levels of deliberate variations in hardness and thickness were made.
- Core tablets at target 100% level made at the final commercial manufacturing site were included.



Composition of the calibration core tablets

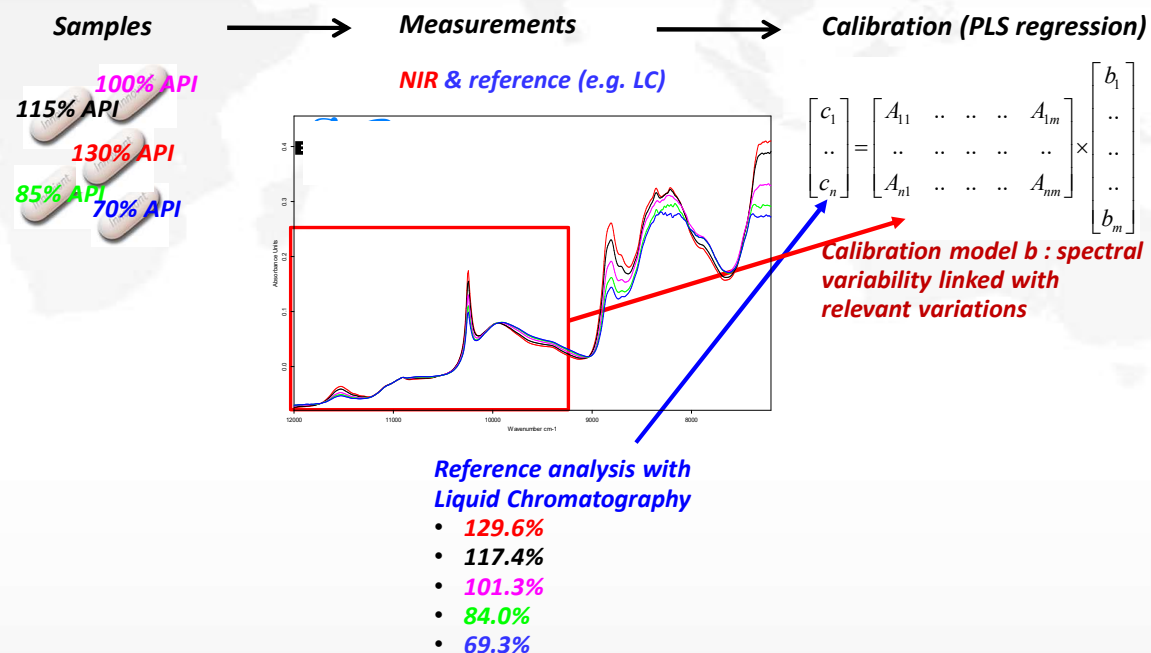
Composition of the calibration core tablets							
Component		% w/w	70%	85%	100%	115%	130%
API			Value API-1	Value API-2	Value API-3	Value API-4	Value API-5
Excipient 1			Value Ex1-1	Value Ex1-2	Value Ex1-3	Value Ex1-4	Value Ex1-4
Excipient 2			Value Ex2-1	Value Ex2-2	Value Ex2-3	Value Ex2-4	Value Ex2-5
Compression Force							
Target	Hardness (N)		Value H1	Value H2	Value H3	Value H4	Value H5
	Thickness (mm)		Value T1	Value T2	Value T3	Value T4	Value T5
High	Hardness (N)		n.a.	n.a.	Value H6	n.a.	n.a.
	Thickness (mm)		n.a.	n.a.	Value T6	n.a.	n.a.
Low	Hardness (N)		n.a.	n.a.	Value H7	n.a.	n.a.
	Thickness (mm)		n.a.	n.a.	ValueT7	n.a.	n.a.

Table:

Example 2: NIR Spectroscopy for Assay

Model development – Calibration model

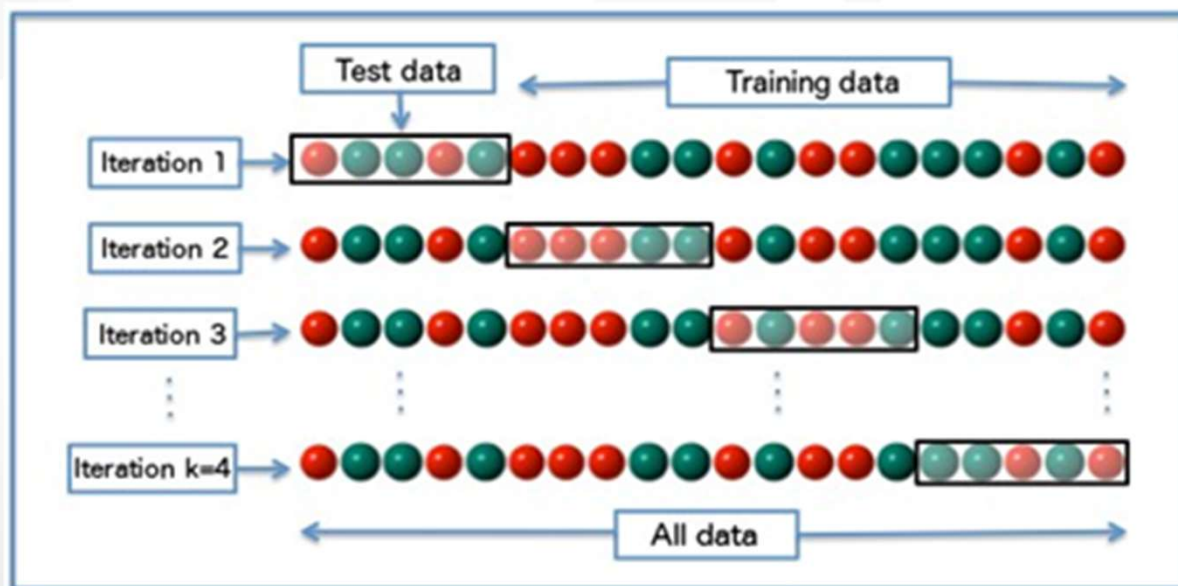
- For the calibration set, each tablet was measured by NIR spectra prior to High-Performance Liquid Chromatography HPLC (reference analytical procedure).
- Other variations like multiple instruments, sample positions and day-to day variation were included in the calibration model design.
- Vector normalization followed by Savitzky-Golay first derivative was used to pre-process.
- 3 partial least squares (PLS) factors were justified based on the model performance.



Example 2: NIR Spectroscopy for Assay

Model development – Internal testing

- Internal testing was conducted with part of the calibration data set in a rotational manner.
- A leave 5-out approach was used as exemplified below.



Example 2: NIR Spectroscopy for Assay

Model development – Robustness

- Other variations assessed as part of robustness were; drug substance lot and particle size, excipient lot, tablet relaxation, tablet moisture content, environmental temperature and environmental humidity.
- A data quality check was implemented, based on the determination of the Mahalanobis distance as well on the residual with associated thresholds.

Example 2: NIR Spectroscopy for Assay

Model validation – Same as Table 3 in module 7 NIR

Performance characteristic	Validation study methodology	Validation results
Specificity/ Selectivity	<p><u>Absence of interference:</u></p> <p>Comparison of drug substance spectrum and the loading plots of the model</p> <p>Rejection of outliers (e.g., excipient, analogues) not covered by the multivariate procedure</p>	An overlay of spectra of drug substance, a core tablet and a placebo tablet are made. Furthermore, plots of the regression coefficients and the relevant PLS components as a function of wavenumbers are reported. Out-of-scope samples are challenged and rejected by the model. Specificity/selectivity was adequate.
Precision	<p><u>Repeatability:</u></p> <p>Repeated analysis with removal of sample from the holder between measurements</p>	Relative standard deviation (RSD) of 1.6% at target level (100%). Repeatability was adequate.
Accuracy	<p><u>Comparison with an orthogonal procedure:</u></p> <p>Demonstration across the range through comparison of the predicted and reference values using an appropriate number of determinations and concentration levels (e.g., 5 concentrations, 3 replicates)</p> <p>Accuracy is typically reported as the standard error of prediction (SEP or RMSEP)</p>	RMSEP of 2.3%. Accuracy was adequate.
Reportable Range	<p><u>Response:</u></p> <p>Demonstration of the relationship between predicted and reference values</p> <p><u>Error (accuracy) across the range:</u></p> <p>Information on how the analytical procedure error (accuracy) changes across the calibration range, e.g., by plotting the residuals of the model prediction versus the actual data</p>	69.3%-132.9%. A linear response, with a correlation coefficient r of 0.998 is obtained. A plot of the residuals of the model prediction versus the actual data was provided. The response was found to be linear across the reportable range.
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<p><u>Deliberate variation of parameters</u>, e.g.,</p> <p>Chemical and physical factors that can impact NIR spectrum and model prediction should be represented in data sets. Examples include various sources of drug substance and excipients, water content, tablet hardness, and orientation in the holder</p>	Variability within and between instruments, tablet hardness and thickness variability, moisture content of tablets, batch-to-batch variability, drug substance particle size variability, tablet relaxation, sample position variability, tablet composition, and environmental conditions of temperature and humidity were successfully demonstrated.

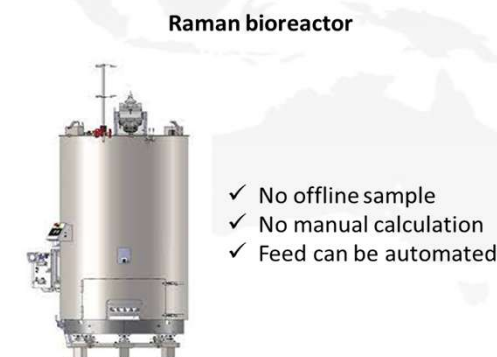
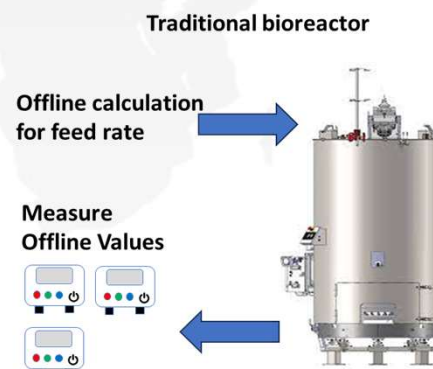
Example 2: NIR Spectroscopy for Assay

Routine use & model maintenance

- The validated NIR analytical procedure is deployed in a routine manner in the commercial manufacturing site.
- Each spectrum measured in routine use will be assessed against the data quality check to determine if the NIR analytical procedure can be deployed or not. When one of the thresholds fails, the NIR model is re-assessed and re-developed and validated if required.
- At justified time intervals, the model is verified by performing a NIR analysis as well as a HPLC analysis on the same tablets of a new commercially manufactured batch.
- The data quality check is tracked and trended to be able to perform pro-active model maintenance.

Example 3: Raman Spectroscopy for Glucose Testing

- Intended purpose (element of the ATP):
 - Continuous monitoring of glucose profile in the bioreactor to control glucose feeding, hereby replacing daily off-line test.
- Technology selection:
 - Analytical technology requirements:
 - No sampling (In-/On-line analysis) and non-destructive
 - Fast analysis (seconds to minutes)



Outcome: In-line Raman Spectroscopy

Example 3: Raman Spectroscopy for Glucose Testing

Model development – Considerations

- Raman spectral data collected on batches at reduced-scale, pilot, and manufacturing scale.
- Batches are designated randomly to the calibration sample set and internal test sample set to avoid any biases to a scale, location, time, etc.
- Sampling bioreactor for reference values is critical.
 - Aligned Raman data with midpoint of sample pull.
 - Offline sample is analysed in a short time frame (within 10 min) to avoid discrepancies.
- Samples are well distributed across the range, and process robustness was built into the model. A design of experiments approach was deployed with component spiking.
- Multiple probes/instruments were deployed to account for instrument variations.

Example 3: Raman Spectroscopy for Glucose Testing

Model validation

Characteristic	Assessed during Validation	Notes
Specificity	+	Compared regression vectors to pure component spectra, The regression will align with increases in glucose concentrations. The model will respond to glucose spiking experiments (to break the time correlation inherent in bioreactor processes).
Accuracy	+	Calculated RMSEP during 3 manufacturing scale batches, across entire range of concentration.
Reportable range	+	Calculated R^2 . Greater than 0.9. Determined during validation for range of glucose concentrations observed during process.
Precision-Repeatability Offline	+	Offline option: Pull 200 mL aliquot from low, mid, high range of glucose. Measure 10 aliquots. Report standard deviation (SD), RSD, and confidence interval.
Precision-Intermediate Offline	+	Offline option: Pull 200 mL aliquot from low, mid, high range of glucose. Measure 10 aliquots with different operators and days. Report SD, RSD, and confidence interval.
Robustness	+	Compare instruments, probes, scale, batches.
Data quality check	-	Model residuals with acceptance criteria.

Contact

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