Guide to TRS100 Analytical Method Development

Agilent

Trusted Answers

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- This document is a tool to assist in the development of quantitative transmission Raman spectroscopic (TRS) methods to the standards required for regulatory approval for release testing
- References the important industrial and regulatory documentation and demonstrates its application in an informative format for the basic user level
- Describes the method development process from feasibility to routine use

Note:

Each quantitative TRS method will be application-dependent and should be treated on an individual basis.

Overall, the method development process is a data-driven scientific process, following logical analytical considerations.

This document addresses the most common processes, problems, and pitfalls of TRS method development.

Key learning outcomes from this guide

- Best practice guidance
- How to make the most of the TRS100
- Optimizing sample creation and data collection
- The decision-making processes for choosing the best method development procedure
- Setting realistic expectations
- The model building process – not a step-by-step guide

Who this is for?

An analytical scientist who may have little or no knowledge of spectroscopy for quantitative analysis

Specialist contributors

Thank you to Phil Doherty of Process Analytics for Section 6 on method life cycle and Acorn Regulatory for Section 7 on making regulatory submissions. This is based on their successful experiences providing support for TRS analytical method submissions to regulatory authorities. Their contact details can be found in Section 8.

Foreword

The main purpose of this document is to serve as a guide for using transmission Raman spectroscopy for quantitative pharmaceutical analysis. From feasibility assessment through to model building, validation, filing of methods to regulatory bodies and life cycle management.

While every effort has been made to prepare a comprehensive roadmap from the perspective of the FDA and EMA processes, it should be acknowledged that the regulatory requirements of any relevant authority should be confirmed before starting development work. It is the responsibility of the end user to be aware of current practices and requirements for the relevant competent authority (CA) and align their efforts to meet these requirements for approval. Approval is entirely at the discretion of the CA. This guide is not intended to be exhaustive, but it should provide a valuable framework for users beginning their transmission Raman spectroscopy journey and help achieve a successful method submission.

1. Introduction

Transmission Raman spectroscopy (TRS) is used for quantitative measurements of oral solid dose forms, for example, tablets and capsules. The primary application of the TRS100 instrument is content uniformity (CU) testing, a quantitative measurement of individual samples to calculate the range of dose strengths within a pharmaceutical batch, resulting in an acceptance value.

TRS is complimentary to traditional analytical techniques for CU testing, for example, HPLC or UV-Vis. TRS requires no sample preparation, no solvents, is nondestructive and takes seconds per measurement.

TRS measures the whole intact sample and gathers information about the Raman active ingredients within the bulk of the sample. The Raman spectrum of a sample is generated by laser illumination and is analyzed by a chemometric model to obtain a quantitative measurement.

By contrast, LC uses chromatographic separation to obtain a response from the analyte of interest. An LC peak's area can be compared to that of a calibration standard to obtain a quantitative measurement. In simple terms, a TRS spectrum generates all the component information at once, whereas LC separates the component information by time. See Figure 1.

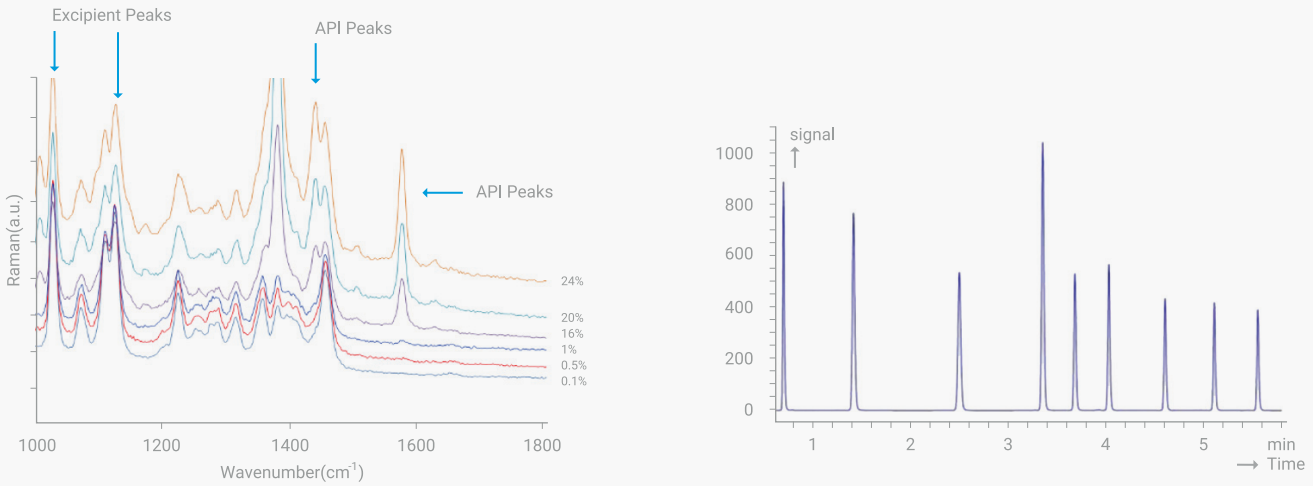


Figure 1. Comparison of a transmission Raman spectrum showing 1,000 to 1,800 cm-1 region (left) and an HPLC chromatogram (right).

Method development of a new analytical procedure can be a significant amount of work. But this can be navigated by checkpoints and progress defined as milestones to guide both the developer and the regulator though the process to a successfully developed method. For TRS, this can often result in a significant saving in product testing time, associated costs (people, consumables, solvents, waste) and the potential for increased testing throughput. See Figure 2.

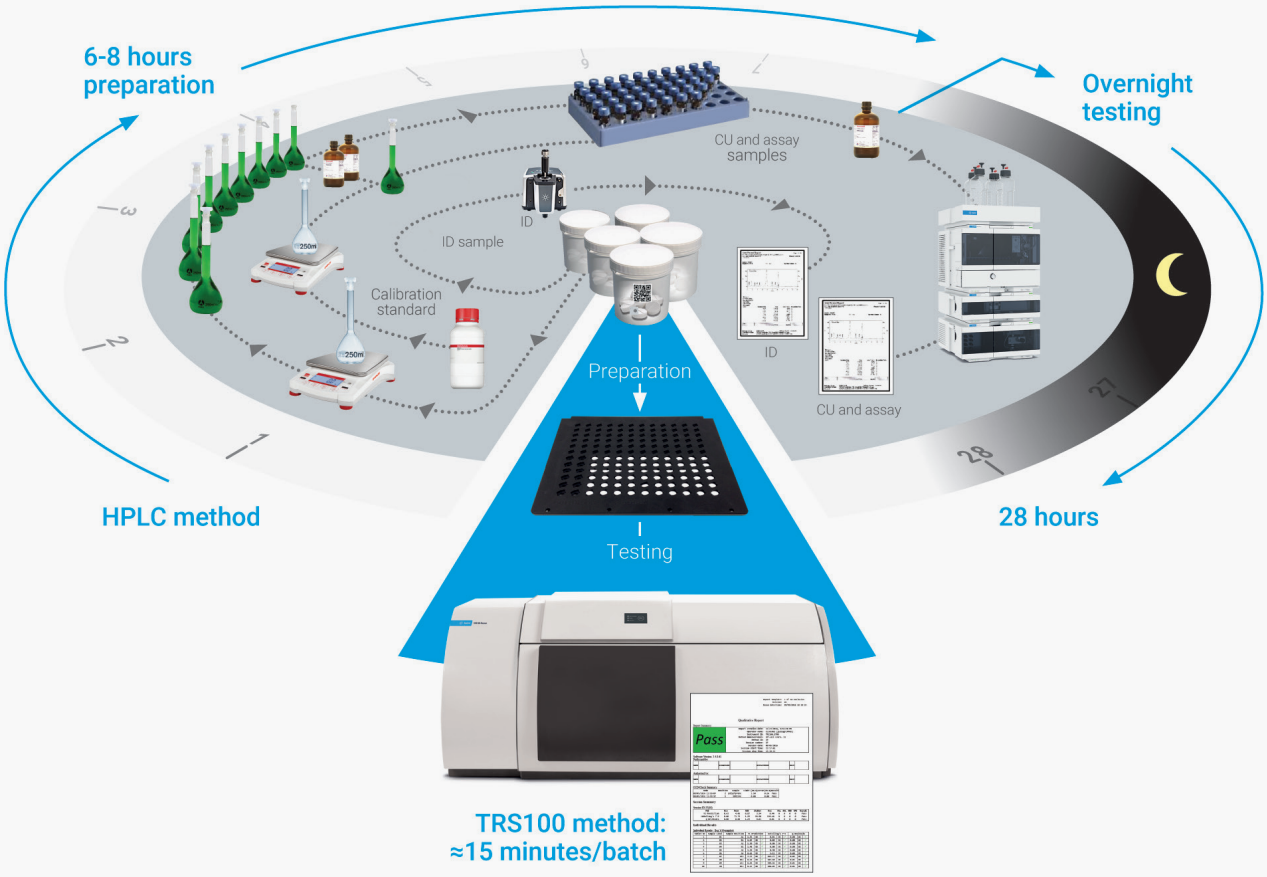


Figure 2. Schematic of TRS100 as a complimentary alternative to HPLC for content uniformity testing, illustrating a more efficient workflow with the TRS100 compared to HPLC. Fewer steps are required and less resources, materials, and time.

Note:

The method development process and success of a given TRS application will always be application-dependent.

1.1 Method development considerations

The TRS method development process assumes that a method is being developed to use as an alternative to another analytical technique. A TRS method is often used as an alternative to HPLC and UV analytical methods. The exact method development process may vary but should consider:

- Design requirements
- Risk assessment
- Method development
- Method validation criteria
- Continuous monitoring and improvement

In development, it is necessary to quantify the accuracy, linearity, and precision for both the reference method and the TRS method to make a meaningful comparison, as per regulatory guidance [1]. By appropriately randomizing real-world variables, such as instrument, operator, and day for both methods, the success criteria for the equivalent method can be demonstrated by comparison.

In quantitative transmission Raman spectroscopy, the method is calibrated using the primary method's results. TRS models are reliant on the primary method and can potentially predict the primary method's result, usually HPLC. Because the secondary method is trained using the primary method, the prediction errors are a sum of both the primary and secondary methods. This is expected and must be explained and allowed for in the acceptance criteria.

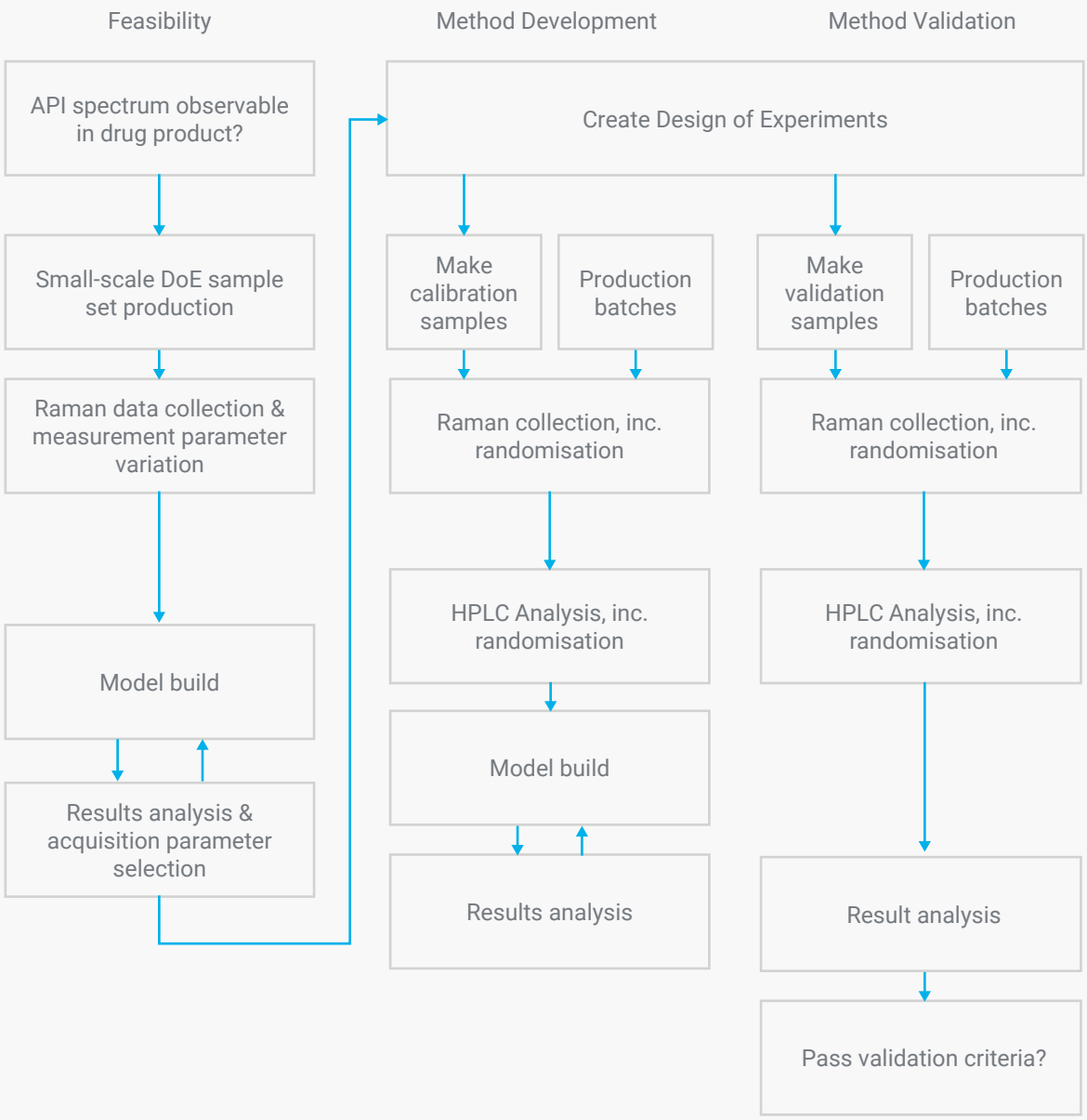


Figure 3. Example of a method development process from feasibility, method development, and method validation.
Note: this does not capture the prior design risk requirements and risk assessment or the continuous monitoring and improvement aspects.

1.2 Measurement of success

To test that the method is successful it must be assessed against suitable validation criteria, which should be established before method development begins.

USP Chapter <1225> Validation of compendial procedures [2] provides definitions and general guidance on validation criteria. ICH Q2R1 Validation of analytical procedures [1] also provides definitions.

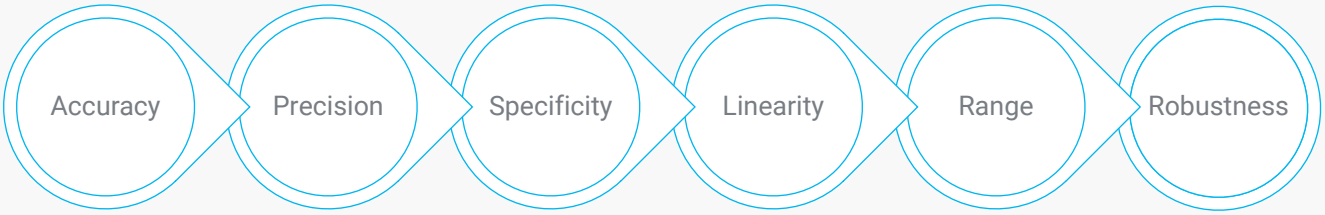


Figure 4. Overview of ICH Q2R1 criteria used to measure the success of TRS method development. Each of these will be covered in further detail in later chapters. The exact limits set will be application-dependent.

1.3 Overview of life cycle

All data-based analytical methods require a maintenance strategy.

Method maintenance is a process to regularly assess the method's ability to correctly predict the reportable results, and a plan to adjust or rebuild the model if necessary. The plan should include strategies for monitoring model diagnostics and action plans in response to deviations or planned changes. Method maintenance is detailed in many of the referenced documents.

Each method is accompanied by a description of the critical elements that need to be monitored during the method life cycle. Processes are referred to in several reference guidance listed in the recommended reading section.

The literature and guidance provide recommendations rather than a rigid framework. Any given application may require unique tests/procedures if they are scientifically justified and evidence if is produced that supports the proposed rationale. The process contains cyclical steps where iterative changes may be made, enabling the flexibility to adapt and improve.

From the relevant guidance and literature, the TRS method development process can be categorized into four key steps. See Figure 5.

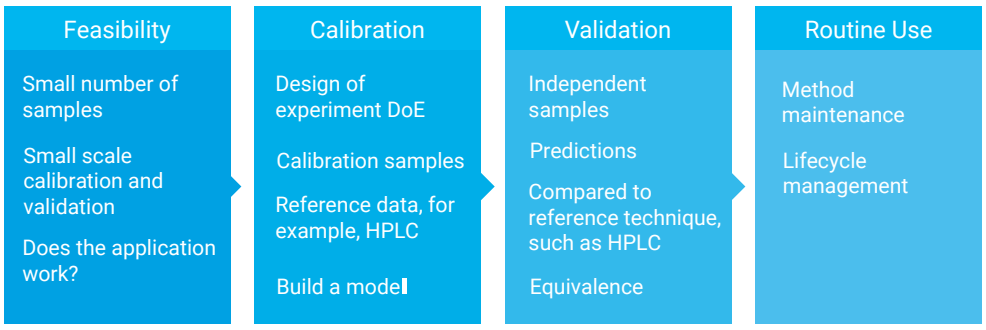


Figure 5. Schematic of the four main stages of the TRS method development process.

2. Fundamentals

This section is an overview of the key points that differentiate TRS from other spectroscopic techniques. See recommended reading at the end of the document. [3]

2.1 Raman spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique. The spectrum obtained provides a chemical fingerprint of the compounds being analyzed. Raman spectra have sharp, distinctive peaks. These peaks correspond to molecular vibrations of the chemical functional groups.

Raman spectra of pharmaceutical products are complex, each ingredient typically has many Raman peaks that combine in number and intensity to provide the spectrum of the whole sample. As well as Raman features, the spectra may exhibit fluorescence contributions. Fluorescence is an additional response from the sample to the laser radiation, characterized by a broader underlying signal. See Figure 6.

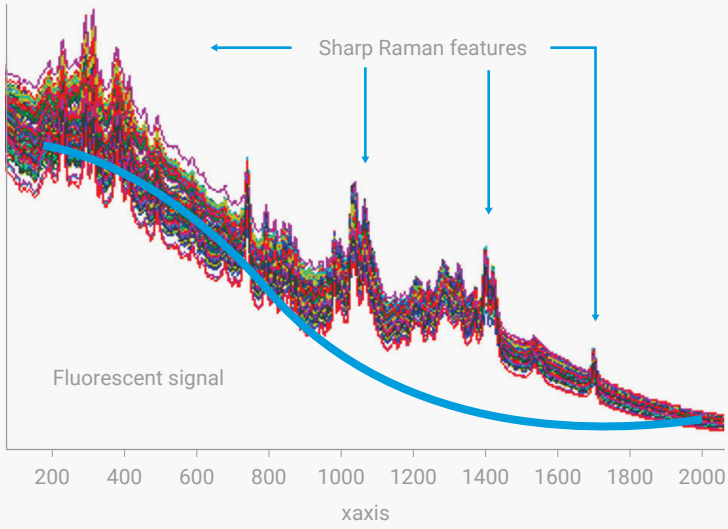


Figure 6. Example of TRS spectra, identifying sharp Raman peaks and a broad fluorescence component.

Transmission Raman spectroscopy is a measurement through a material that scatters light. The material is often nontransparent, for example, a pharmaceutical tablet. The laser is located on the opposite site of the sample to the detector, resulting in the Raman signal being collected from the bulk of the material. Conventional Raman instrumentation, benchtop, probe, and handheld devices operate in a back-scatter geometry, where the laser is delivered to the sample on the same side as the signal is collected.

Back-scatter Raman generates a comparatively more intense signal, but the signal is biased to the surface. Transmission Raman is a weaker response due to scattering losses throughout a sample, but the signal originates throughout the bulk of the material. Figure 7 shows the creation of transmission Raman photons as the laser light scatters through the sample, which helps when measuring thicker samples.

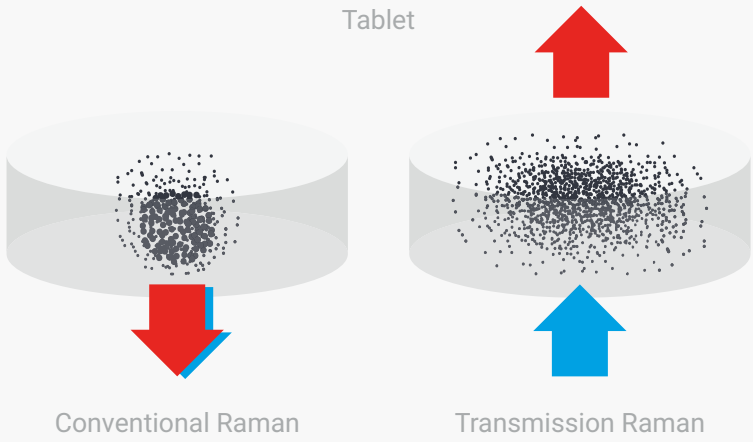


Figure 7. Comparison of the Raman sampling volumes of transmission Raman and conventional Raman.

The amount of transmission Raman signal measured by the detector varies depending on amount of sample, thickness, scattering processes, and more. Unlike absorption methods, the intensity of Raman signal versus thickness in TRS exhibits a maximum signal, around 2 to 3 mm of sample thickness. This unintuitive result is due to the balance between elastic and inelastic Raman scattering. [4]

Figure 8. Transmission Raman increases with thickness, then decreases due to absorption effects.

For more information on transmission Raman, see [5–7].

2.2 Chemometrics

Chemometrics is the science of extracting information from chemical systems by data-driven means. Chemometrics is inherently interdisciplinary, using methods frequently employed in core data-analytic disciplines, such as multivariate statistics, applied mathematics, and computer science, to address problems in chemistry, biochemistry, medicine, biology, and chemical engineering. Wikipedia [8]

Chemometrics was originally defined as the chemical discipline that uses mathematical, statistical, and other methods that employ formal logic to accomplish two objectives: (1) to design or select optimal measurement procedures and experiments, and (2) to provide the maximum amount of relevant chemical information by analyzing chemical data. More specifically, "chemometrics" has come to mean the application of multivariate methods for the analysis of chemical or related data, although the algorithms in question may be used to extract information out of almost any measured data, regardless of origin.

USP <1039> Chemometrics [9]

Transmission Raman spectra of pharmaceutical products are complex; the spectra contain many peaks and features relating to the complex mixture of ingredients. HPLC is a separation technique that measures the peak area of a response (typically UV) of an API. This is a univariate technique.

'Uni' = one 'Multi' = many
Raman spectra contain many peaks from each of the Raman active compounds in the sample, which overlap to form a complex, information-rich analytical starting point. Chemometrics (multivariate analysis) allows us to deconvolute/trend/analyze this complex data type.

Common multivariate techniques

Model Type	Explanation
PLS: Partial Least Squares	Quantitative model – this is the workhorse for TRS100 method development
PCA: Principal Component Analysi	Qualitative tool to look at inherent patterns and trends in the data
PCA: Principal Component Analysi	Classification model that returns most probable class

For further reading, see [10,11]

2.3 Units

For CU testing the individual assay results can be expressed in %LC (label claim) and then used to calculate the acceptance value (AV) for content uniformity.

For transmission Raman spectra the absolute spectral signal intensity varies with the amount and thickness of material. To get a measure of the concentration of API, the Raman spectra are normalized to a relative intensity. We measure %w/w of sample. With TRS, we usually only care about the relative changes in the Raman spectra.

Figure 9. The units in TRS method development are %w/w which, when multiplied by the tablet mass result in the dose, and when divided by the target dose reach a %Label Claim value.

3. Step 1: Feasibility - finding a suitable candidate

Feasibility is about finding a suitable candidate before committing to full method development.

3.1 What is a good sample?

Some samples are more suitable than others for quantitative analysis using TRS. An important variable is how well a sample diffusely scatters the laser (elastic scattering) and then generates a Raman signal (inelastic scattering).

Tablets work very well; they diffusely scatter the laser light though the bulk of the sample, generating Raman signal from a large volume of the sample. Tablets of the same type are generally very consistent in terms of size and density.

The coating may affect the amount of Raman signal obtained in comparison to an uncoated core. The extra layer of material may absorb laser light (especially highly colored – reds/purples) and very thick samples may increase path length and reduce Raman signal.

Powders and capsules work very well. These powder-based samples have the tendency to vary in sampled volume due to powder shifting in the capsules/bag/vial, so the sample-to-sample absolute Raman signal may vary. This variation is handled by normalization during preprocessing of the spectra.

Liquid suspensions and clear liquids are the poorest sample types for TRS. The laser goes straight through liquid samples and opportunity for scattering the light and generating Raman signal is reduced. The viability of success will also depend on the sample, for example, a pure solvent or substance will likely work well but aqueous solutions will be difficult due to water.

The Raman spectrum of water is at much higher frequencies than excipient and APIs and can be effectively ignored. However, any effect of water on the sample constituents should be evaluated and considered.

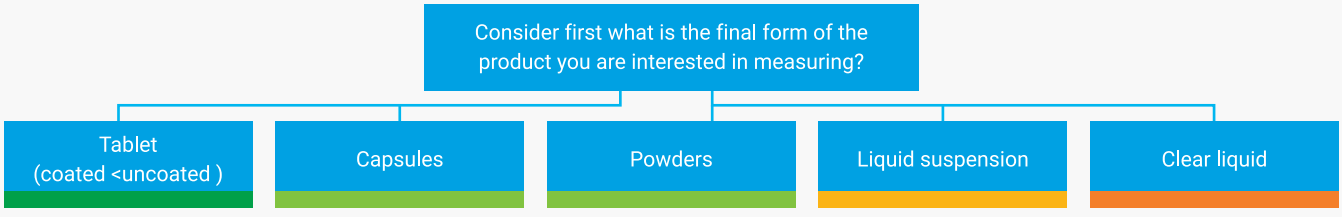


Figure 10. Schematic for the success of transmission Raman with different types of pharmaceutical.

3.1.1 What is in the product you are scanning?

The next consideration is the formulation. The detection limit of TRS is ≈1%w/w for APIs. This is, of course, application dependent and LOQs approaching 0.2%w/w have been reported [12]. In general, APIs are good Raman scatters; some are better than others and occasionally APIs are inherently fluorescent. The more API in a formulation the easier it will be to model.

In cases where the Raman signal is dominated by the API, the excipient is harder to see. In TRS, quantification of any one ingredient is related to the quantities of the other ingredients, as the TRS measure is a %w/w of the whole formulation. The API:excipient ratio determination is therefore related, so the excipient must also be quantifiable. High dosage API products containing a small amount of excipient can be challenging applications.

The bulk excipient will also affect the ability to see, measure, and quantify the API %w/w. In general, lactose-based formulations work better than cellulose, predominantly due to the fluorescence from cellulose based excipients.

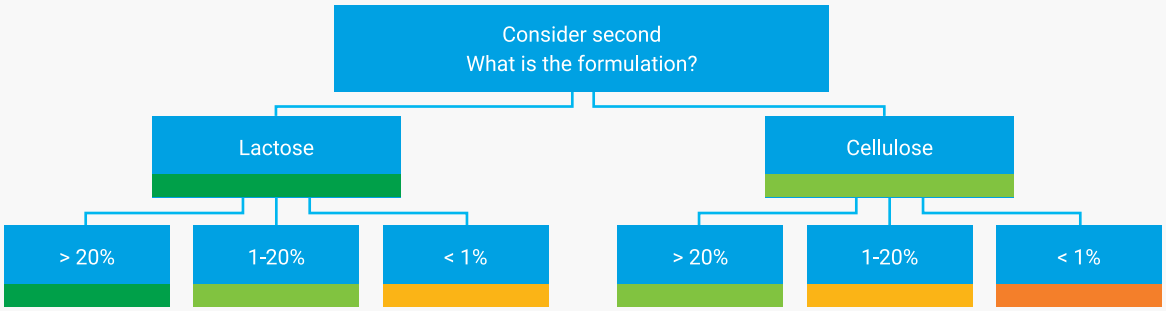


Figure 11. Schematic for the success of product formulation, consideration of main excipient, and %w/w of API.

Note:

For powder samples in bags, we recommend filling the bag evenly and consistently, and removing lumps. A homogeneous powder layer is best. One inch2 bags hold ca. 100 to 700 mg.

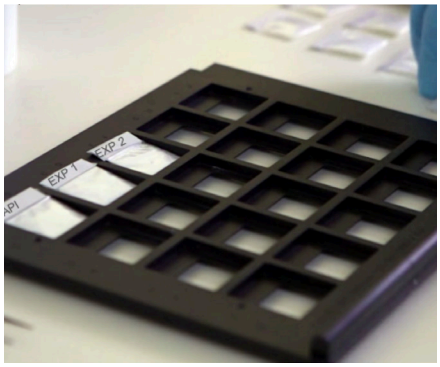
Filling the bags with the same mass of material makes it easier to judge the Raman scattering ability of each excipient and API.

Given all of this information, the best way to gauge success is to scan it and see. This is quick to do.

3.2 Finding the right sample

For definitive results, the approach of scan it and see will answer the previous presumptions.

To answer the question 'Can we see the API in the final product?', Raman spectra of the final product, API, and excipients should be analyzed.



Note:

At this early stage, it is good practice to save and print pure component spectra to learn and recognize key peaks from the most common pharmaceutical ingredients.

3.2.1 Example: Scanning excipients and final product

The example in Figure 12 is what might be expected from scanning pure API, the excipients, and final product. This API spectrum is typical; sharp well-defined peaks with little to no fluorescence. Peaks ~ 1600 to 1800 cm-1 are characteristic of carbonyl C=O or aromatic benzyl functional groups. In general, this chemical functionality is usually present in APIs and not in excipients. The region of 1600 to 1800 cm-1 can therefore be used as a quick identification of pharmaceutical samples to see if API features can be seen in the spectra.

Cellulose-based excipients, such as L-HPC and MCC, are fluorescent. Lactose is a good Raman scatterer with sharp well-defined peaks.

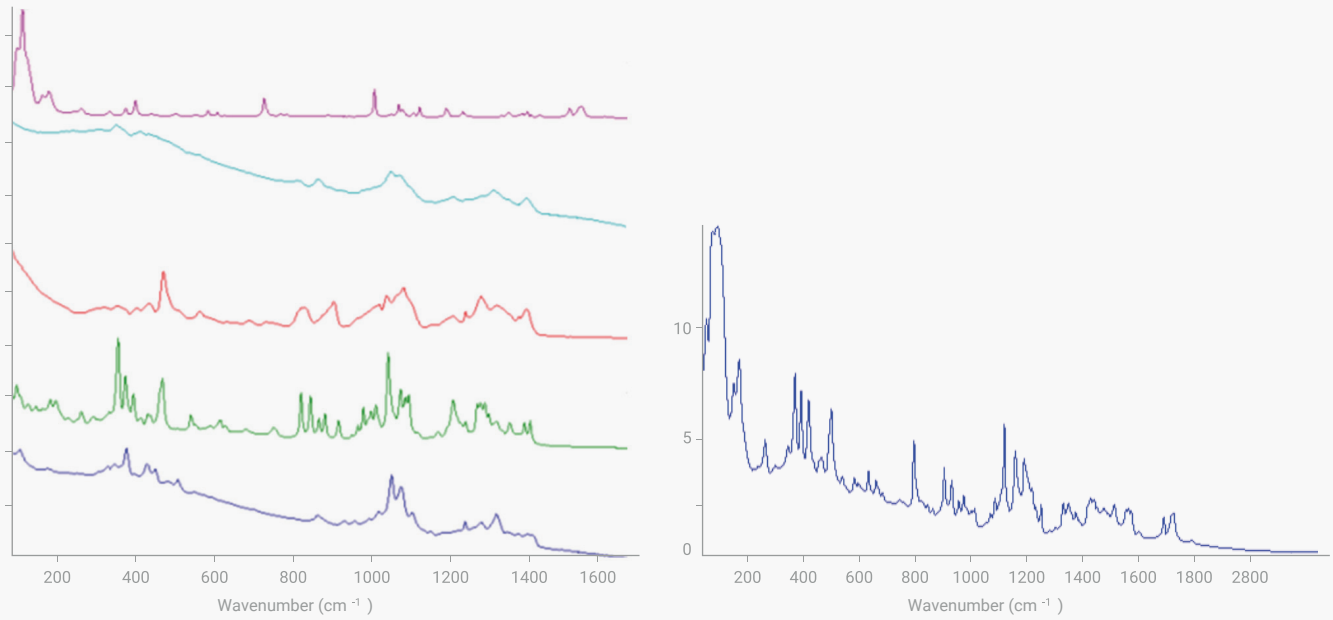


Figure 12. Example of pure component spectra and a final product, tablet spectrum. Look for API peaks in the tablet spectrum to identify API in the final product.

Note - Spiking study:

Spiking studies **are not** robust calibrations. Spiking studies should be used for feasibility only. Spiking studies vary just one substance so are limited in terms of scope and viability.

3.2.2 Example: Spiking study

The above example is relatively trivial; the API can be clearly observed in the tablet spectrum. If the API peaks cannot be seen clearly, if there is overlap with your excipients, or if it is difficult to distinguish which peak comes from which substance, is there anything you can do?

A spiking study may give some answers.

One approach could be to take the final product, for example, 10 tablets, grind these up using a pestle and mortar and spike in some API over a reasonable concentration range, as demonstrated in the worked example
in Figure 13 and Figure 14.

3.2.2.1 Worked example: Spiking study

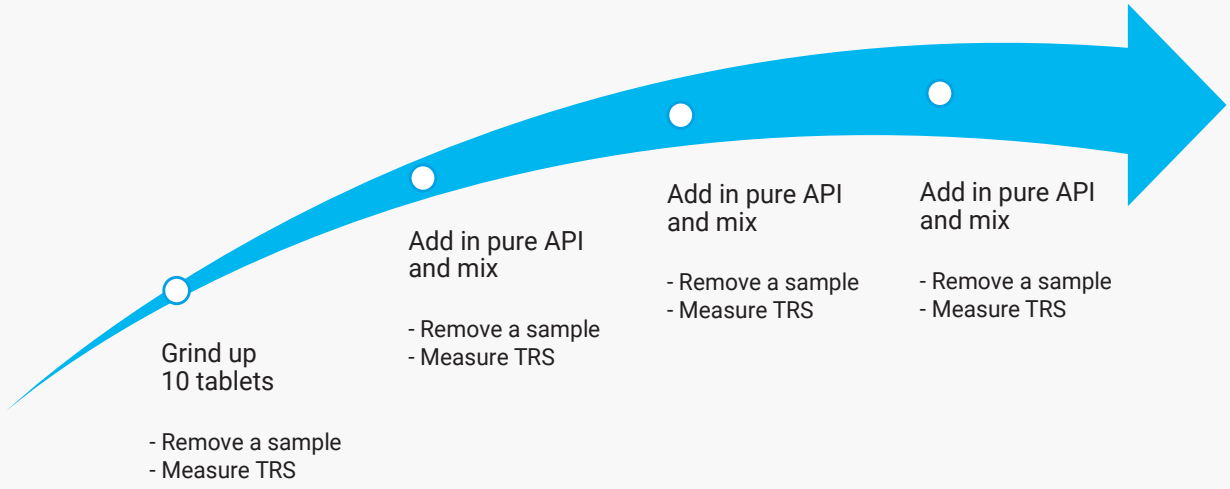


Figure 13. Suggested spiking study procedure.

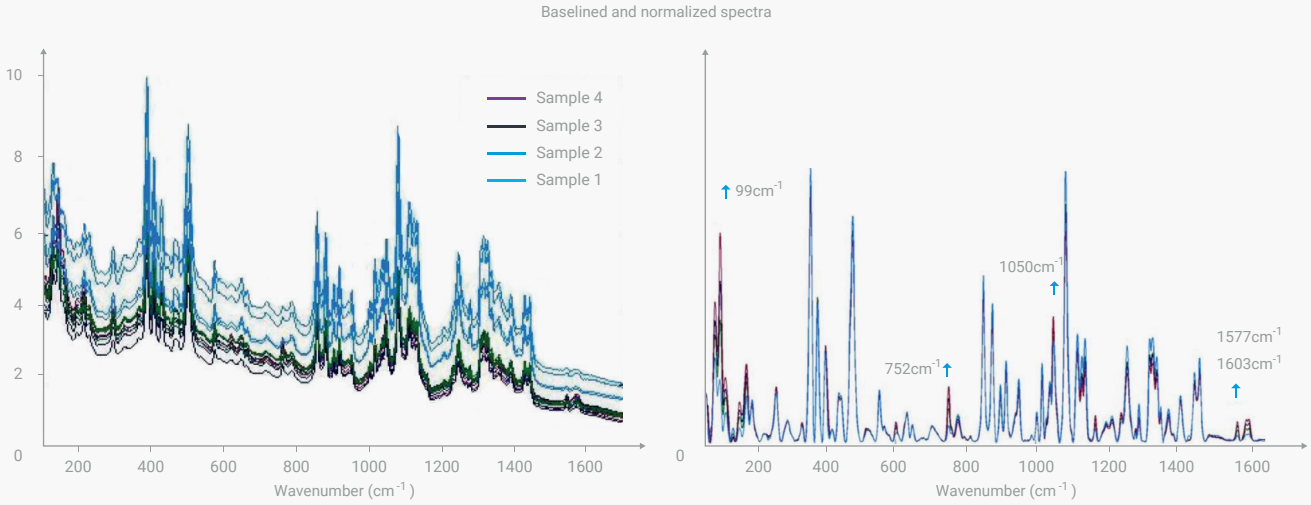


Figure 14. Spiking study example of raw spectra and baselined and normalized spectra indicating spectral variation due to an increase in API concentration. Seeing variation of API in the spectra indicates a good candidate for TRS method development.

3.2.3 Other considerations

Each application may be subtly different and require different considerations when scanning. Some sample-specific considerations are listed below.

- Sample presentation:
  - Tablets
    - Scanning the center of the tablet is normally preferred.
    - A teardrop shaped tablet is likely scanned off center where the highest volume of the tablet is.
    - Embossing/printing can affect measurement, make sure to determine side to measure from.
    - Bilayer tablet – determine side to measure from for consistent measurement.
  - Capsules
    - Ends – scan different ends to determine the effect.
    - Center – going through two layers of a capsule.
- Number of scan positions
  - One scan position is typically preferred.
- Large dosage units - scan across multiple positions and ensure a consistent signal and is representative of the bulk.

4. Step 2: Calibration

The aim of the calibration phase is to build a successful calibration model to predict unknown samples. This calibration will be tested with the validation phase.

4.1 Making samples

Calibration is a familiar term in the analytical sciences; a set of known values that can be used to provide a measurement scale to compare with unknown values.

With HPLC, calibration uses samples with known quantities of the compounds of interest (separated by chromatography from other analytes) in a reference standard, which is compared with peak intensities of the unknown quantities in the measurement sample.

With spectroscopic techniques, such as Raman, a complex mixture of ingredients in the drug product are measured. Separation from the other product components is not possible, therefore the calibration samples need to represent the final product and all its components. **Since the concentrations of each component are relative to each other, variance of all the ingredients is needed.**

We recommend a design of experiment (DoE) approach, in which each individual ingredient is varied independently of the others. This creates a robust design space and the targeted product should sit in the center. A robust model is insensitive to minor changes in production variables. This should be familiar to anyone with experience of developing quality by design (QbD) methods and submissions.

Typically, a DoE may range from eight to 25 samples. A DoE that varies four variables can be visually represented by a cube, this is known as a central composite design. The variables could be API#1, API#2, excipient#2, with the main excipient#1 used as the mass balance. A single variable may be a combination of singular ingredients, such as an excipient blend of the minor components. The production or target samples are represented by sample 7, and these sit right in the center of the design in Figure 15.

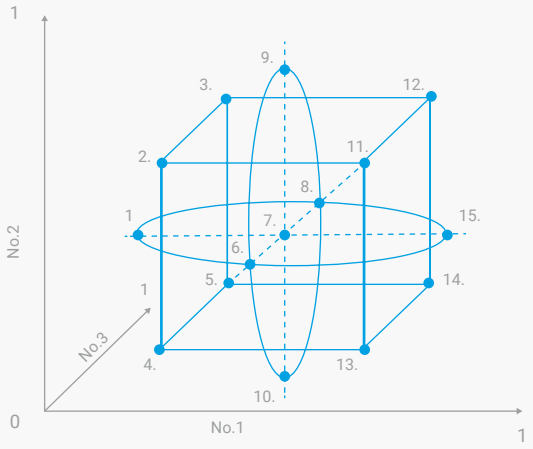


Figure 15. Pictorial representation of a central composite design DoE showing concentration variance of three components.

A calibration sample set that only varies one ingredient, for example, API, creates a very narrow design space, which is fine for feasibility but will be sensitive to small changes in other process variables. This approach is **not** recommended for robust calibration sample design.

4.2 Which DoE? DoE decision tree

There are no set rules for the type of DoE that must be followed. Again, application dependence is the only set rule in this whole approach.

Below is a potential decision-making process to aid you.

NB. More samples can be added to the calibration depending on model performance; it can be an iterative process and calibrations can be modified based on the results. Also, it is sometimes easier to make all, or the majority, of the samples needed in one go to save time.

For complex formulations, it may be possible to combine the smaller and weaker Raman active ingredients into a 'preblend' to reduce the number of individual ingredients requiring dispensing, and to mitigate errors of weighing out small quantities of material, as suggested in Figure 16.

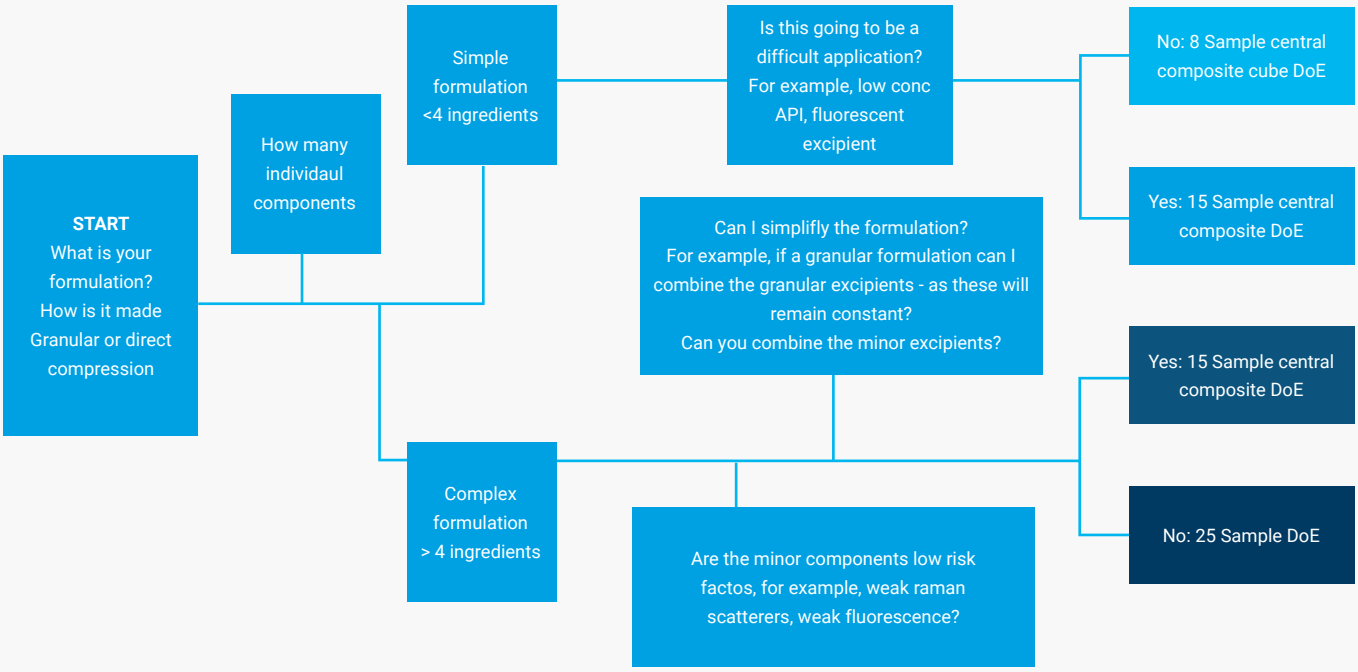


Figure 16. Decision tree for design of experiments.

4.3 Making samples

The best calibration performance is achieved when the calibration samples are a close match to the samples that you are predicting. There may be formulation factors such as size, shape, and matrix effects such as compaction force and particle size. Again, there are no set rules and each application will have a different set of important variables.

4.3.1 Scanning powders before compressing into tablets

After the calibration powder blends have been formulated and before tableting, scanning these blends using TRS is a quick process for a 'look and see' as to the success of sample preparation. With each scan taking between 10 to 60 seconds, this may be valuable time spent before effort is expended on tableting.

Results may indicate that more calibration samples are required, or mixing isn't homogeneous within the powder.

4.3.2 Thinking forward to validation

When dispensing and making samples, you could combine this with making your validation samples; there is usually no need to separate this process.

It may be a good idea to make up repeated samples of the same concentration level to prove reliability of sample preparation and help demonstrate accuracy as part of the validation criteria.

4.4 Other factors to consider

As each pharmaceutical product and process is unique, it is important to consider risk factors that may affect the quality of the Raman result. These may include but are not exclusive to those in Figure 17. [13]

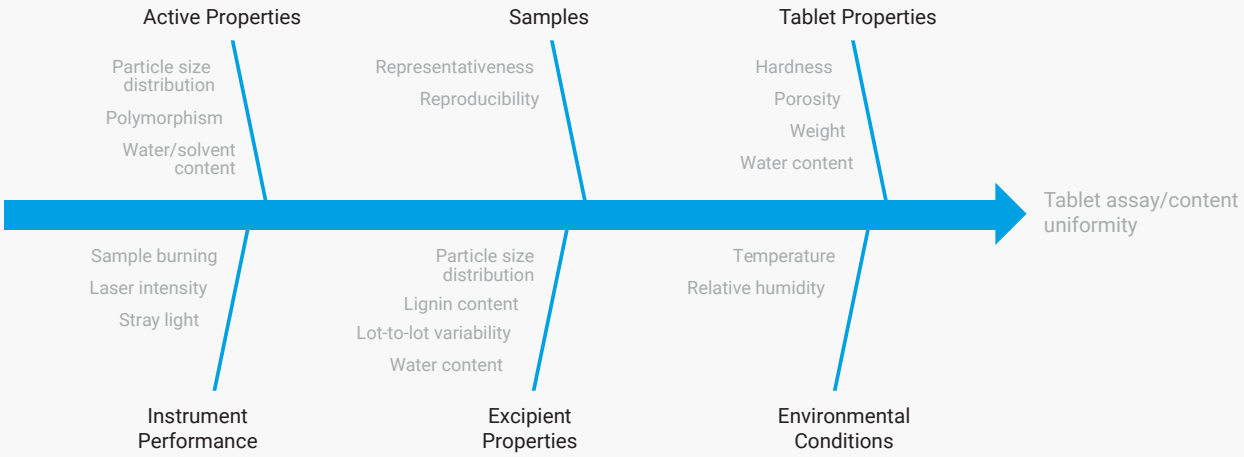


Figure 17. Risk factors to consider and design into your calibration space. [14]

4.5 Measuring samples

Good quality spectra will generate better quality chemometric models and ultimately better results. This section describes the acquisition parameters and suggested choices.

4.5.1 How to scan samples

There are two factors to consider when scanning samples: (1) the optical settings, such as laser spot size and lens collection choice and (2) the acquisition settings, for example, laser power, scan duration, and scan replicates.

4.5.1.1 Optical settings

The TRS100 unit has the option to vary the laser spot size and the lens collection optics, see Figure 18.

The laser spot size options are 2 mm, 4 mm, or 8 mm. Each spot size has the same power but different power densities, with the 2 mm laser spot being more intense than the 8 mm. Altering laser spot sizes varies the volume of sample being analyzed and larger spot sizes reduce the laser intensity for fragile samples, for example, a red gelatin capsule may melt with a 2 mm spot size.

The available lens collection optics are small, medium, or large. An increase in the optics size will increase the area of the sample being analyzed. Any changes to the laser and lens optic settings should be matched with the size and type of sample being analyzed, ensuring the sample illumination isn't outside the tablet boundary.

The effect of lens and spot size should be investigated as part of the method development process. Experience suggests a 4 mm laser spot size and medium

optics work well for most tablets and capsules. Also, multiple acquisitions at different sample positions can help increase effective sampling volume.

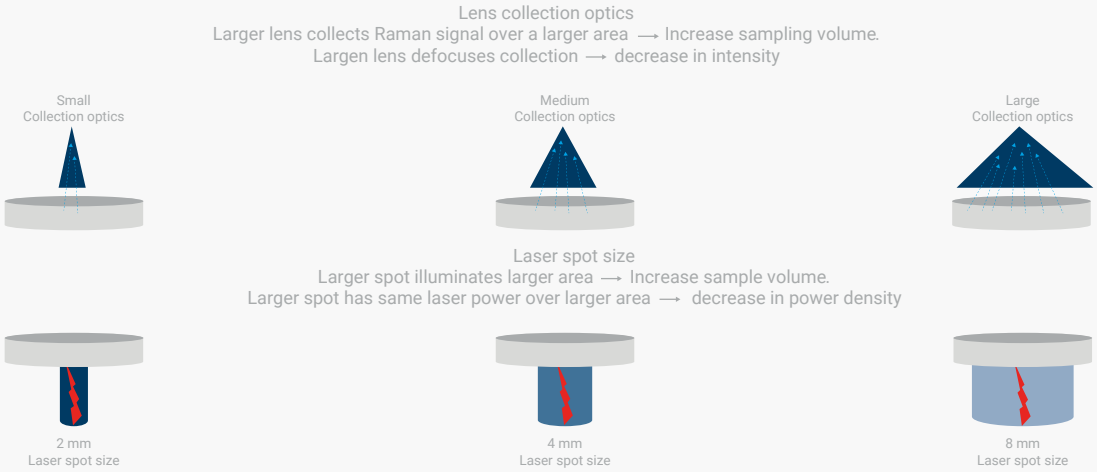


Figure 18 . Schematic of the laser spot size and lens collection optics.

4.5.1.2 Acquisition settings

The maximum laser power of the TRS100 is 650 mW, which can be reduced in the software if needed. Camera exposure time and number of accumulations can also be configured in the software and should always include multiple accumulations to avoid gamma ray artifacts.

The saturation limit of the detector is around 65,000 counts per accumulation; however, it is recommended to avoid using the maximum working range and to limit to a working range of ca. to 40,000 counts by using the following workflow when optimizing acquisition settings:

- Scan sample at 0.65 W for 1 second and one accumulation
- Observe spectrum
- Set exposure time:
  - If < 40,000 counts, increase the exposure time
  - If > 40,000 counts, reduce the exposure time
  - If > 40,000 counts at 0.01 exposure time, reduce the laser power
  - Repeat until the appropriate exposure time is achieving ~ 40,000 counts
- Increase number of accumulation
  - Must be > 3
  - A good estimate for total scan time (exposure x accumulation) is approximately 10 seconds for most samples
  - The optimum number of accumulations will always be application-dependent
  - It may be useful to measure for 10 seconds – observe signal-to-noise, rescan at ~ 60 seconds and look for significant improvement
  - Model performance of data collected under different number of accumulations may indicate if the signal-to-noise is limiting in the model space

4.5.1.3 A note on saturation

Raman peaks are usually stronger in the lower wavenumber regions; this is due to the detector efficiency (quantum efficiency or QE) or the camera's ability to translate photons into electronic signals, with weaker Raman intensities being observed at higher wavenumbers.

Quite often, Raman peaks of interest for pharmaceutical products have distinctive peaks at higher wavenumbers ~ 1600 to 1800 cm-1. To improve signal-to-noise in the higher wavenumber region and 'see' API peaks, it can be acceptable to increase the exposure time and purposefully saturate lower wavenumber regions, as long as the prediction model uses only signal within the working range.

Although the detector range is higher, spectral intensities of > 40,000 counts should be avoided as the detector response can be nonlinear and could add errors into model building and prediction. The spectral range can be selected as part of the model building process.

Saturation limit should be observed in the raw spectra that has not had the Y-axis intensity correction/green glass correction applied.

4.5.1.4 Repeated scanning - A note on photobleaching

Fluorescence is a competing emission process of a pharmaceutical sample that has been irradiated by a laser (Section 0). On repeated scanning, a fluorescent component may exhibit successive reduction of the fluorescence intensity – this effect is called photobleaching. This may affect your Raman measurements and model predictions. There are ways to mitigate and reduce the effect of photobleaching, for example, you may include multiple measurements of the same samples in a model to train the model to recognize the effect of photobleaching.

The repeat pocket and repeat tray function within the software can assist with this type of analysis.

4.5.1.5 Repeated scanning - Homogeneity scanning

It is possible to scan across multiple locations of the same sample – this can inform you of the spectral homogeneity of the sample. It may be particularly useful for a powder blend uniformity application, or if agglomerates are a known issue for a given application. This may form part of a feasibility strategy.

4.6 Building models

There are many text books and lecture courses dedicated to chemometric analysis and model building [3,10,11]. The intention here is not to reproduce that work but to summarize the most common workflows and decisions that are considered as part of the transmission Raman method development process.

From previous sections, where the formulation was considered to make DoE and sample choices, the feasibility scans may help determine the DoE for calibration and validation. In any case, model-building can be iterative and may lead to making/including more samples.

The output of the model building process is not necessarily fixed – it is possible to change, update, or modify a model with new included or excluded data, subject to being scientifically valid. Calibration and validation measurements and sample preparation can often be done together to avoid wasting time and expense.

As a general note, TRS data is different to NIR data in form and structure. Raman peaks are generally sharp and highly specific for each analyte. The experience of building calibration models for NIR is not always the best approach for TRS and caution should be taken when applying NIR principles to TRS data.

4.6.1 How to build a model

4.6.1.1 Sample selection

Note:

It is possible to load a multi Y block and predict multiple constituents in one model.

Samples included in the model should be representative of the sample that will be measured/predicted following the guidance in earlier sections and should adhere to QbD/ DoE principles.

Terminology:

- Spectra loaded into the model will become the X block.
- Concentrations loaded into the model will become Y block.

4.6.1.2 Spectral region selection

There are several approaches to selecting the spectral region(s) to be included in the model. Spectral selection may be the whole spectrum or smaller region(s) of interest. Saturated regions should always be excluded from the model building process. The limit for TRS100 is 40,000 counts per accumulation. Spectral region selection can follow an iterative process of making a change > building a model > seeing how it affects results > making a change.

Suggested process:

- Start with the full spectral region.
- "Top and tail": < 200 cm-1 is often saturated, > 1900cm-1 often contains no Raman peaks.
- Next focus on key areas where you have API **and** excipients. It is important to have both API and excipients represented as %w/w is used for quantification. A ratio is needed between at least two (ideally more) Raman active ingredients.

4.6.1.3 Chemometric preprocessing choices

Preprocessing involves manipulating the spectral data to optimize model performance. Preprocessing should maximize the spectral differences of interest, for example, API concentration variation, and minimize other influencing factors, such as thickness.

For transmission Raman spectra, three preprocessing steps are routinely performed.

Disclaimer:

This is not prescriptive; different applications may require different options for best performance. It is also likely that several preprocessing options may give similar model performance – there may not be one best option but a selection to choose from.

Note: Spectral preprocessing **hint**

It is good working practice to plot the baselined and normalized spectra and color according to API concentration to visualize spectral variation and compare to pure component spectra.

If you can visualize spectral banding, which is associated with API concentration, this gives a strong indication that the application will be successful.

Step 1: Baselining

This removes the fluorescence background, which is generally of no interest. For example, Whittaker baselining, first derivative, second derivative.

Step 2: Normalization

This removes overall relative intensity variance, as we are interested in %w/w variation. This minimizes differences due to things like sample thickness. For example, normalize, SNV, MSC.

Step 3: Mean center

This is often applied to Raman data; it removes the average of the entire data set from each spectrum. This removes any common features and leaves the differences.

4.6.1.4 How many latent variables?

As part of the model building process, you will have the option to change the number of latent variables to build a model.

Latent variables = principal components = factors. Terminology is often used interchangeably.

Latent variables are spectral responses that the model uses to correlate the concentrations given (Y block) to the spectra (X Block). The latent variables should relate clearly to the spectrum of the compound being measuring. This is a clear benefit of TRS over, for example, near infrared (NIR) spectroscopy where components are not always clearly resolved.

As few latent variables should be used as possible to prevent over fitting. Often have less or equal to N+1 latent variables, where N = the number of factors varying in your DoE. Again, this is an area where TRS's specificity can be an advantage over NIR.

The more latent variables in the calibration model, the more the apparent performance will improve, as any noise from the experiment becomes incorporated into your model. However, the ability of that model to predict new, independent samples is likely to suffer. This will be tested with model validation and independent samples.

4.6.1.5 Model optimizer

As should become obvious from the information in this guide, model building is repetitive and iterative. There are tools within chemometric software packages to speed up this model optimization process. The best practice is to interpret the model output with good scientific judgment and not to solely rely on the computational output; in short, make sure what you are doing makes scientific sense.

4.6.1.6 Cross validation

Cross validation is a process that occurs within the calibration phase and is generally automated in chemometric software packages. It involves building models with various iterations of included/excluded samples and seeing how that model predicts the remaining samples. The purpose of this is to test the model's robustness by adding and removing samples.

There are various algorithms available in software for performing this; however, default options often suffice.

4.6.1.7 When is enough, enough?

When striving for the best possible model, it can be hard to determine the endpoint since models can always be tweaked and changed. The aim is for a robust and reliable model that adequately meets your testing requirements. Model statistics are a part of this decision-making process. Validation of the model using independent samples allows you to complete the model development and test it against the validation criteria.

It is always possible after analyzing validation samples to revisit the calibration process either with physical samples, measurements, or model rebuilding of existing data.

4.6.2 How to interpret a model's performance

The output of chemometric modeling provides a myriad of graphs and numbers to interpret. The key factors are described in the next section.

4.6.2.1 Model statistics

An important graph to demonstrate model performance is shown in Figure 19. This plot shows the 'measured' values of a sample as described in the imported Y block (concentrations) and the 'predicted' value that the model generates. A linear fit between these two indicates that the model can adequately correlate spectra with the given concentrations. A measure of this is R^2 An $R^2 > 0.95$ is generally considered a good value.

Root mean square error values **(RMSE) a**re a measure of model error.

RMSEC: root mean square error of calibration

RMSECV: root mean square error of cross validation

RMSEP: root mean square error of prediction (only obtained with independent validation samples)

RMSEC and CV should be low and be roughly be the same. If RMSEC ≈ RMSECV this indicates that your model can predict calibration samples robustly when calibration samples are removed.

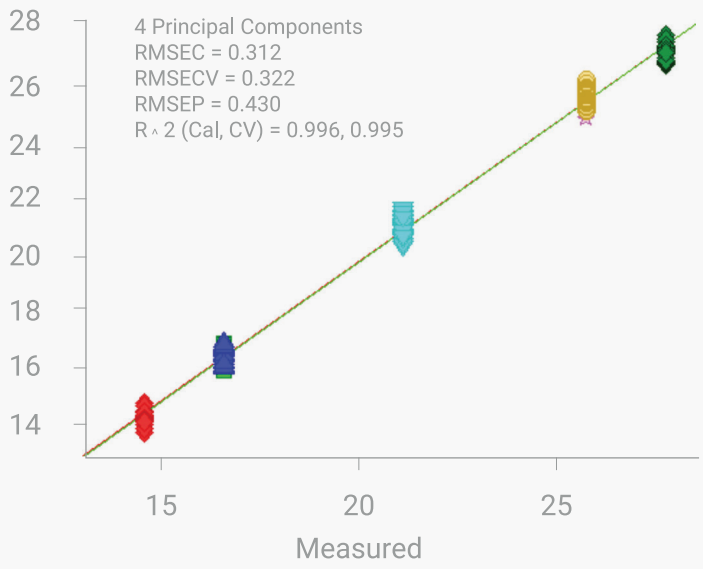


Figure 19. Example of a PLS model output.

Hotelling and Q-residuals are other model statistics that are used to judge model performance and sample quality within a calibration sample set. The two statistics describe the relationship/similarities of samples within the calibration space. See Figure 20.

Hotelling: Describes the relationships of samples with respect to information already in the calibration space, such as extremes of ingredient concentration.

Q Residuals: Describes the relationships of samples with respect to information not in the calibration space, for example, a feature not seen in the calibration such as noise of an unknown compound.

As good working practice, most of the calibration samples should sit in 95% of your calibration space; outliers are acceptable, and they shouldn't be the central concentration point. This is expected because, as part of the DoE, concentrations of all ingredients will vary away from the target center point.

Hotelling and Q-residual plots are a good way of identifying spectral outliers of an entire sample, or individual samples. Good sample preparation and data acquisition should show individual scans of the same tablet from the same DoE sample clustering together. Good working practice for sample exclusion should be fully justified, for example, sample X was friable and fragile. This is because sample X is at the edge of the DoE, containing very low levels of excipient X. Spectra of sample X were outliers in hotellings; as such these have been excluded from the model space because they are not representative of a production quality tablet.

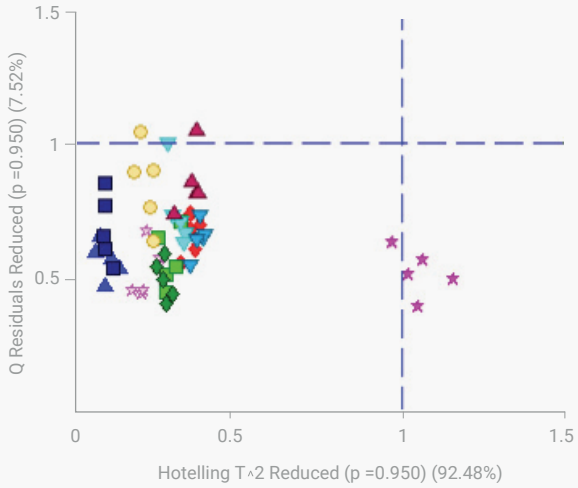


Figure 20. Hoteling and Q residuals plot. The blue dashed lines indicate the limits at 95%. In this example, the pink stars are outliers and sit away from the other samples due to an extreme of one ingredient. Samples of the same color sit close to one another, which is a good qualitative attribute. The remaining samples all sit within or very close to the limits, which is acceptable.

4.6.3 Reference data: Equivalence – HPLC vs Raman errors

What is the Y block? The source of the concentrations used for model building may progress over time. It is common practice to start model building using the gravimetric values for the Y block, this enables a fast result. However, the gravimetric values may not be representative of what's in the sample – a reference measurement may be required.

Consider the example below, where a set of samples at 80% nominal LC are assumed to be identical; however, when individual LC values are added, the actual concentrations range between 76 and 86%.

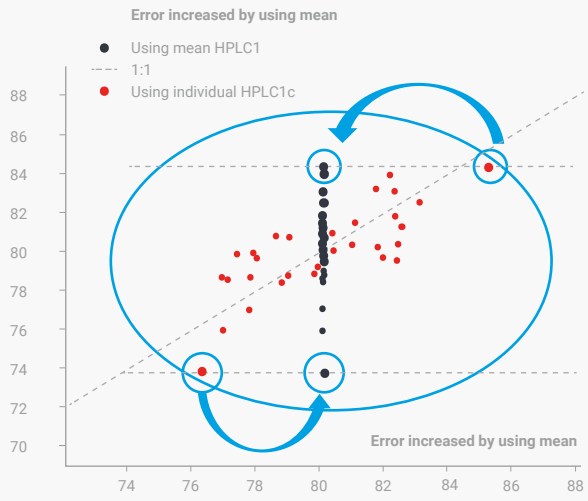


Figure 21. Effect of incorporating HPLC into model development.

The effect on model performance of incorporating HPLC values into a model is shown below in Figure 22. By using HPLC values, the model statistics improve, giving higher R2 and lower RMSECV.

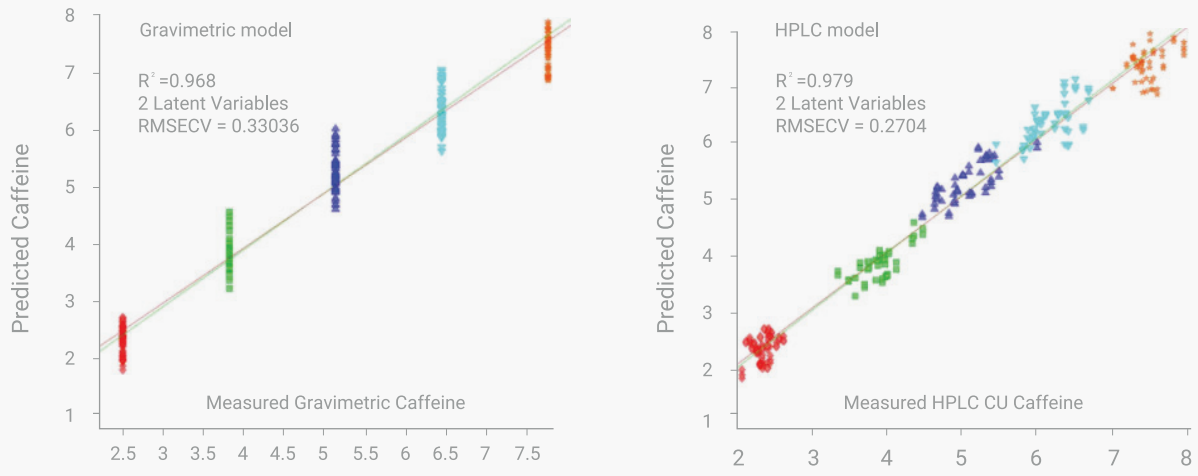


Figure 22. Effect of incorporating HPLC into model development.

Using reference techniques does not **always** improve your model performance. It may come down to what can be done more accurately: making samples up to a known concentration or weighing them accurately.

Errors:

TRS model error = TRS error + reference technique error, for example, HPLC.

Using HPLC errors as the Y block incorporates HPLC errors into the TRS model. This means that the error of prediction will never be less than the HPLC error. A helpful way to look at this is that TRS is predicting the HPLC result in an equivalency model, not the concentration of the API. Therefore, it is often referred to as a secondary method.

Considering the above, the validation criteria for any method should be set appropriately. In a secondary equivalent method, such as TRS, the expected error will be larger than the HPLC method.

Averaging

This is a brief section about best practice for averaging spectra. This may be applicable when looking at the effect of multiple scans across a sample of a powder bag where a single prediction value is required. The best practice is to average the predicted output rather than the spectra in a preprocessing step before model building. This way the model 'learns' a greater amount of uncertainty or noise.

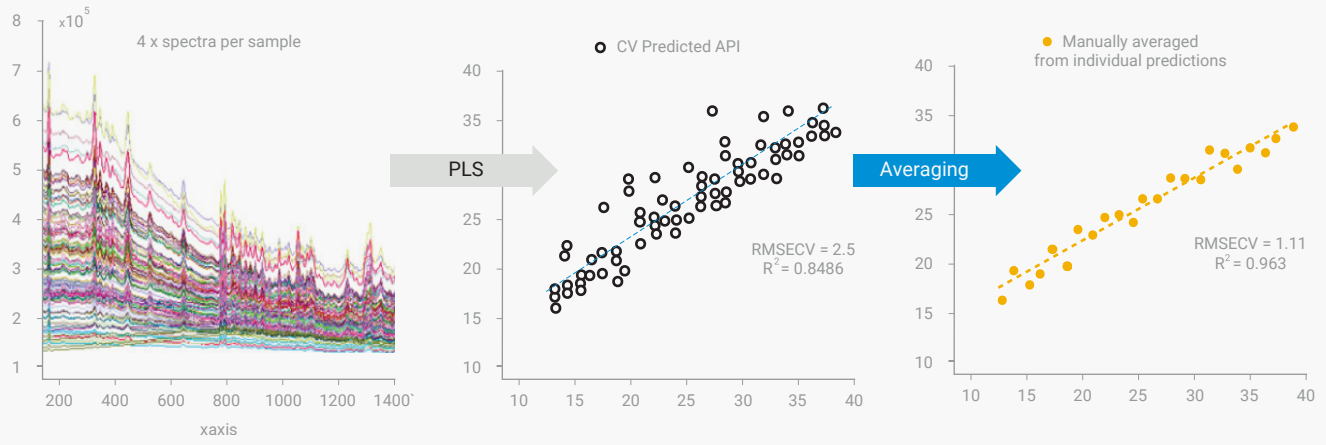


Figure 23. Demonstrating the optimal workflow of averaging spectral information by averaging the predicted output.

5. Step 3: Method validation

Validation is the process of testing the calibration model. It completes the process of generating a robust and reliable calibration model, which can then be deployed for routine use. As with every step in this guide, the exact validation process is likely to be application-dependent and follow slightly different protocols. Best working practices and suggestions will be discussed in this section.

A reminder of how method success is measured and demonstrated is shown in Figure 24.

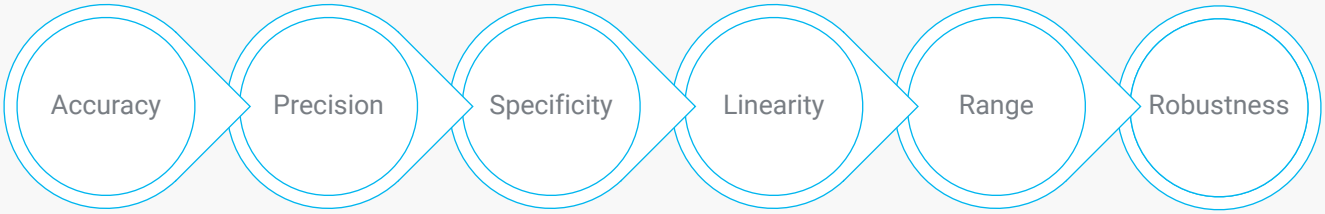


Figure 24. ICH Q2 R1 guidance.

5.1 What samples to use for validation?

To appropriately test the calibration model, suitable validation samples should be used; again, these are likely to be application-dependent.

Validation samples should be independent from the calibration samples. Examples:

- Production samples
- Good samples
- Bad samples
- Samples that span your calibration space and test your model
- Samples that encapsulate expected variation in your production process
  - Capture natural API/excipient/process variation
  - Capture different lots from different time points

5.2 Demonstrating accuracy

Guidance suggests:

'Accuracy should be established across the specific range of the procedure, which would normally be by comparison of the results with the validation reference method.' [14]

An example of the TRS predictive result compared to the primary HPLC method is shown below in Figure 25. It is important that the techniques are compared using the same units, see section 2.3 on Units. Raman methods generate a %w/w result, whereas HPLC generates %LC result per sample result. For comparison, conversion using tablet weight is often required: %w/w x tablet mass = mg active.

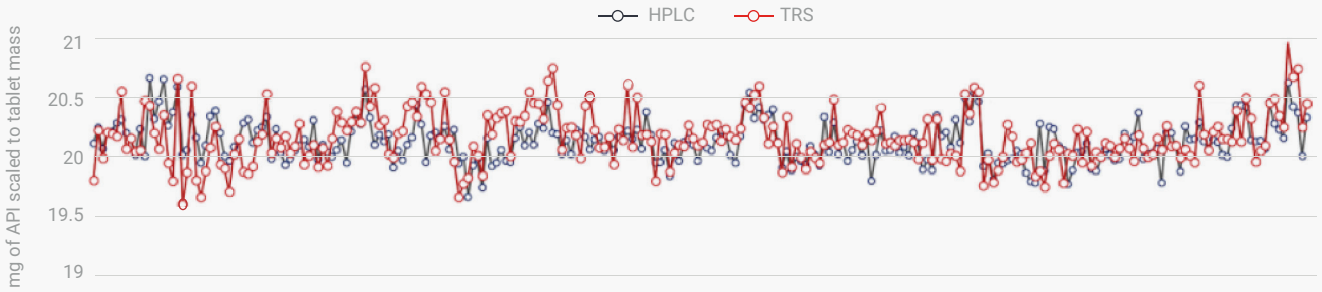


Figure 25. Comparison of TRS predictive output compared to primary reference technique, in this case HPLC.

The key chemometric statistic for prediction errors is RMSEP, root mean square error of prediction, see section 4.6.2.1 Model statistics 0. This metric ensures that the model generalizes well and still achieves the same performance on new, independent data that is "unseen" by the model. The RMSEP values should therefore be compared to RMSEC and CV values to avoid overfitting of the data.

RMSEC ≈ RMSECV ≈ RMSEP

The success criteria for a given application may differ, but could include:

- RMSEP
- Using statistical tests, for example, t test or f test
- Confidence limits

5.3 Demonstrating precision

Guidance suggests:

'Repeatability and intermediate precision should be determined, covering the specified range. [14]

Repeated scanning of samples using TRS is quick and nondestructive, so this information can be readily obtained. It is generally recommended to do this over multiple concentration points and not solely on center point or production samples. Success may be determined by establishing a %RSD variation limit.

Table 1. Example of precision tests.

Precision	Intermediate precision	Interinstrument
Same day	Multiple days multiple analysts	Multiple instruments

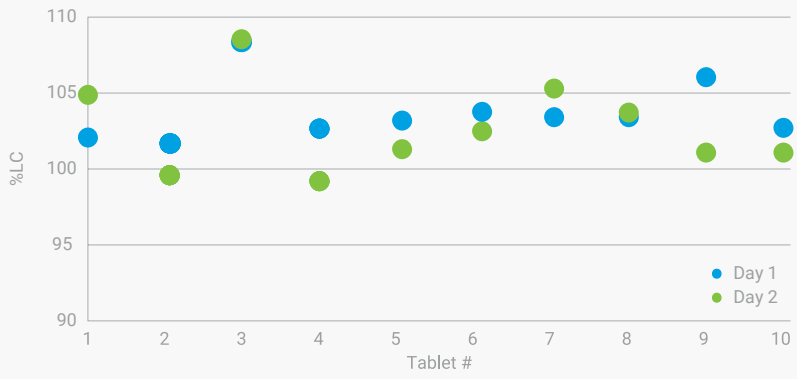


Figure 26. TRS predictions demonstrating intermediate precision, the effect of scanning the same samples on different days by different analysts.

Table 2. Example of data used to establish precision of 10 individual tablet samples over two different days, comparing results to the primary reference technique HPLC.

	Day 1	Day 2	Day 1-2	TRS	HPLC	DTRS - HPLC
1	102.3	105.0	2.7	103.7	98.4	5.3
2	101.8	100.0	-1.8	100.9	97.8	3.1
3	108.0	108.1	0.1	108.1	103.7	4.3
4	102.3	99.7	-2.6	101.0	100.3	0.7
5	103.4	101.9	-1.5	102.6	99.9	2.7
6	103.6	102.8	-0.8	103.2	99.8	3.4
7	103.9	105.2	1.3	104.6	101.6	3.0
8	103.7	103.8	0.1	103.8	100.1	3.7
9	105.6	103.1	-2.5	104.4	101.6	2.8
10	103.0	101.5	-1.5	102.3	101.1	1.2
Average	103.8	103.1	-0.7	103.4	100.4	3.0
RSD	1.8	2.5	0.7	2.0	1.7	
AV	8.0	9.0		8.2	4.5	

Table 3. Example of analytical parameters compared to predetermined acceptance limits for a specific analytical application.

Analytical Parameter	Acceptance Criteria	Result	Status
Accuracy/specificity (Average difference HPLC-TRS)	NMT 5%	Tablet diff. 3.0%	PASS
Precision TRS repeatability intermediate (%RSD)	NMT 5%	Day 1 tablet diff. 1.8% Day 2 tablet diff. 2.5% Average tablet diff. 0.7%	PASS
*specification (AV value)	AV NMT 15	50 mg tablet diff. AV 8.2	PASS

Note:

Alternatively, regression vectors or VIP scores can be used.

5.4 Demonstrating specificity

Guidance suggests:

'A procedure should be able to access unequivocally the analyte in the presence of the other components.' [14]

A Raman spectrum of a pharmaceutical sample is a mixture of all the components. Often, the model development process is interested in only measuring a compound of interest, such as a single API. As discussed in the chemometrics section 0, latent variables are the spectral features that the model has used to generate the regression between calibration and given concentrations. The first latent variable is the most prominent (spectrally, at least) component.

We can demonstrate specificity by comparing the latent variables (ideally the first) to the pure component spectra gathered in the feasibility stage of method development. If these are similar, this demonstrates the correct modeling of the analyte of interest. This is shown in Figure 27.

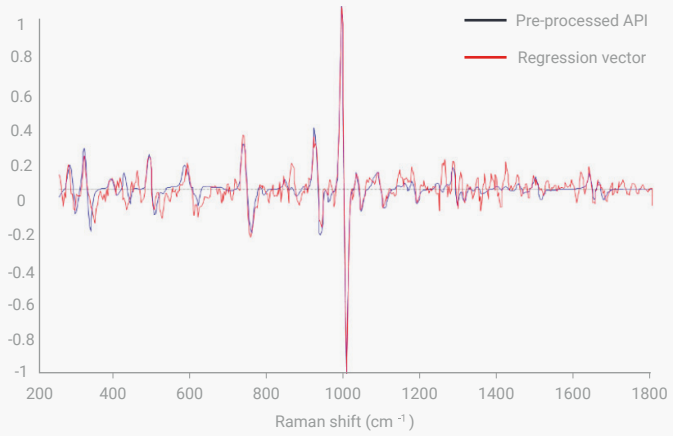


Figure 27. Demonstration of what specificity looks like.

Note - Spiking study :

Note: HPLC often exhibits R2 > 0.99. HPLC linearity is an assessment of the detector's response linearity to different concentrations. By contrast, in spectroscopic techniques it is the comparison between two methods that are being assessed. [15]

5.5 Demonstrating linearity and range

Guidance suggests:

To demonstrate linearity, it is required that the samples in the validation set are distributed across the specified range.' [14]

By plotting the measured vs predicted values, the R2 value indicates linearity across the validation range. R2 > 0.95 is generally acceptable.

5.6 Demonstrating robustness

Guidance suggests:

'Evidence to demonstrate the robustness of the spectroscopic procedure should cover chemical and physical variables, the conditions employed, sampling and sample preparation, as well as variations in the procedure parameters.' [14]

Testing for robustness, as described in previous sections, will be completely application-dependent. Examples include:

- Chemical variation
- Raw material variation
- Sampling
- Sample preparation

An example of robustness to compaction force is shown in Figure 28. The different colors are three different compaction forces. The plot of measured vs predicted (bottom left) indicates that all samples can be predicted well, with R2 ≈ 1 and low and alike RMSC/CV/P values. However, some segregation is observed in the hoteling T2 and Q residual plot (top left). The model recognizes subtle spectral differences between samples of different compaction forces. A user may decide to use the Q residuals as limits or alarms for compaction force variation.

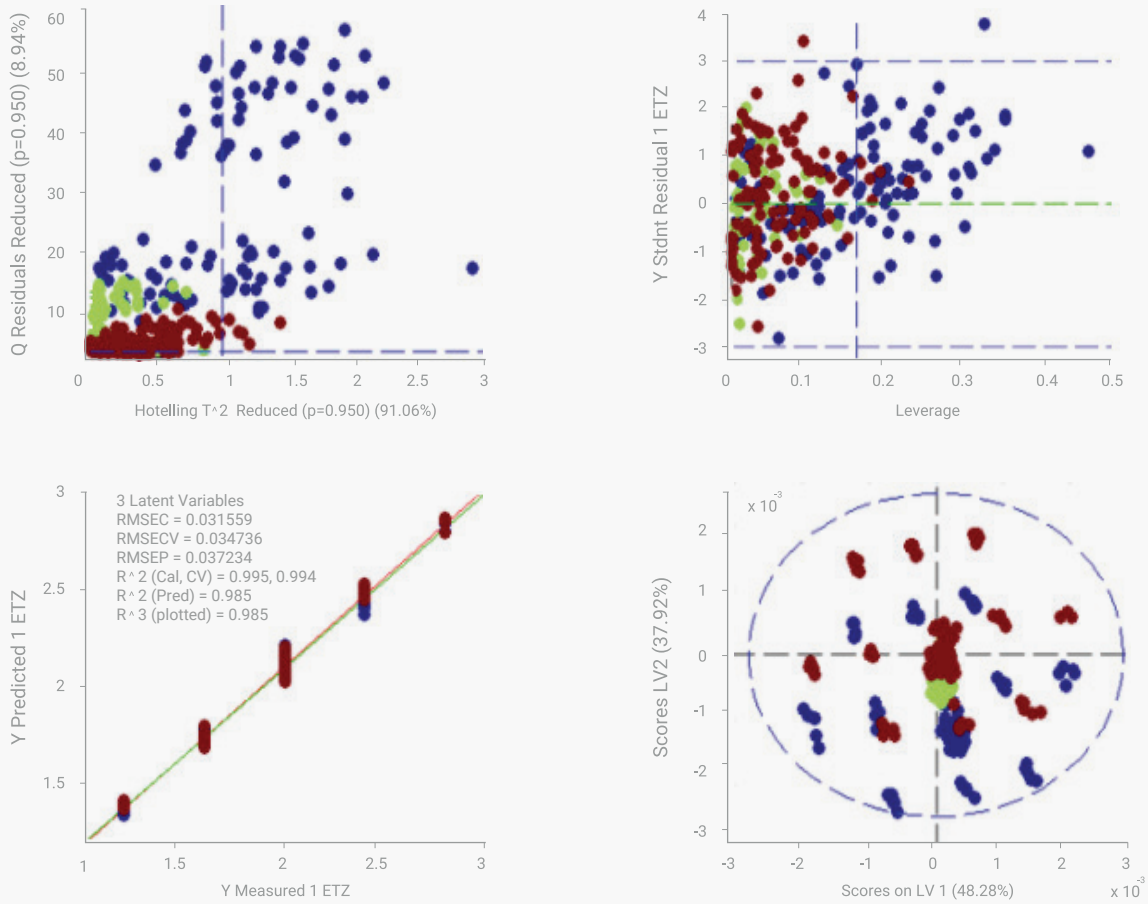


Figure 28. Example demonstrating robustness with variation of compaction force.

Another example of robustness, this time to excipient supplier, is shown in Figure 29. Different excipient suppliers (different colors) predict similarly, with R2 ≈ 1 and low and similar RMSC/CV values.

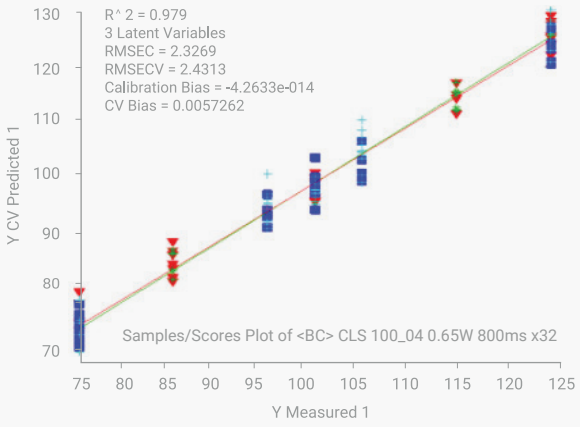


Figure 29. Demonstrating excipient supplier robustness.

5.7 What if my model isn't good enough?

The process of method development is iterative. If a certain set of parameters/ calibration samples has not produced adequate results, improvements may be possible.

Possible changes that could be made:

- Add more samples into the calibration: This may be bringing in some samples of known variation, for example, samples with different compaction forces.
- Remove samples: Samples should only be removed from the calibration if scientifically justified, for example, if they are fragile and do not represent production quality material.
- Change measurement settings, for example, increase exposure time if the model performance is signal-to-noise limited.

In all decision-making processes for Raman method development, sound scientific logic should be adhered to, according to QbD principals. If a thorough risk analysis at the start of the process has been considered, all expected process variation and samples should have been considered, mitigating for the need to go back and make more samples.

6. Step 4: Method life cycle

6.1 Model maintenance, update, and life cycle

Regulatory guidelines recognize that spectroscopic methods may evolve over time, after the initial regulatory application, because of planned changes (managed by internal quality procedures through an implemented change control policy) and unplanned changes.

6.1.1 Changes within the scope of the method

Changes to the spectroscopic method that are within the method scope should be validated as per this procedure, but do not require a regulatory variation. Such changes should be risk assessed and any identified risk mitigated through good scientific, manufacturing and engineering practices and controls. Examples of such changes include addition of spectra in the calibration model, adaptation of sampling devices, and software upgrades.

Changes to the manufacturing process, such as new raw materials and suppliers, that are within the scope of the method should be assessed by carrying out parallel testing on at least one batch. If the spectra after the changes pass the spectral check and the results pass the specified precision, accuracy, and recovery acceptance criteria, the spectroscopic method need not be changed. Otherwise, the method should be updated with spectra of samples made after the manufacturing process/raw materials/suppliers change. Whether the spectroscopic method requires changes or not, the results of the assessment should be documented.

6.1.2 Changes outside the scope of the method

Changes to the spectroscopic method that are outside the method scope should be validated as per this procedure and do require a regulatory variation. These changes can include extension of the method's specified range, changes to the data collection parameters outside of the registered TRS method, or changes to the specification limits.

6.1.3 Parallel testing

Parallel testing (testing of samples by TRS, then testing of the same samples by the reference method for product release) can be carried out for TRS methods that have been submitted for regulatory approval, while the submission is being reviewed.

6.1.4 Periodic reference method/TRS comparison

The developed TRS model's performance should be monitored as per internal mechanisms for analytical target performance profiling. To verify the continued agreement between the reference method and the TRS method, testing of the same samples should be performed, at least annually, by both TRS (first) and the reference method. Such periodic testing can be carried out on invalid samples (for example, first running of the process) or on QC release samples.

The samples tested by both the reference method and TRS should comply with the accuracy and acceptance criteria of the validation protocol. If they do not, an analytical laboratory event investigation should be performed to understand the root cause and assign corrective and preventive actions.

6.1.5 Handling of out of specification and out of trend results

In the event of a TRS out of specification and/or an out of trend result, this should begin a standardized procedure for investigation of root cause. If the atypical result is found to be caused by a previously unknown input to the model, this should trigger a model maintenance and revalidation cycle to incorporate this new experience and update the TRS model.

This overall process is summarized in the flowchart in Figure 30.

6.1.6 Major instrument repairs

Following major instrument repairs, the TRS method should be re-assessed by carrying out parallel testing on at least one batch. If the spectra after the repairs pass the spectral check and the results pass the precision, accuracy, and recovery acceptance criteria, the spectroscopic method need not be updated. Otherwise, the method should be updated with spectra of samples made after the repairs. Irrespective of whether the spectroscopic method requires updating or not, the results of the assessment should always be documented. Instrument repairs constitute a change within the scope of the method.

6.1.7 Method transfers between instruments

If more than one TRS instrument is present onsite, methods should be developed using both instruments to ensure that this variability is included within developed TRS models.

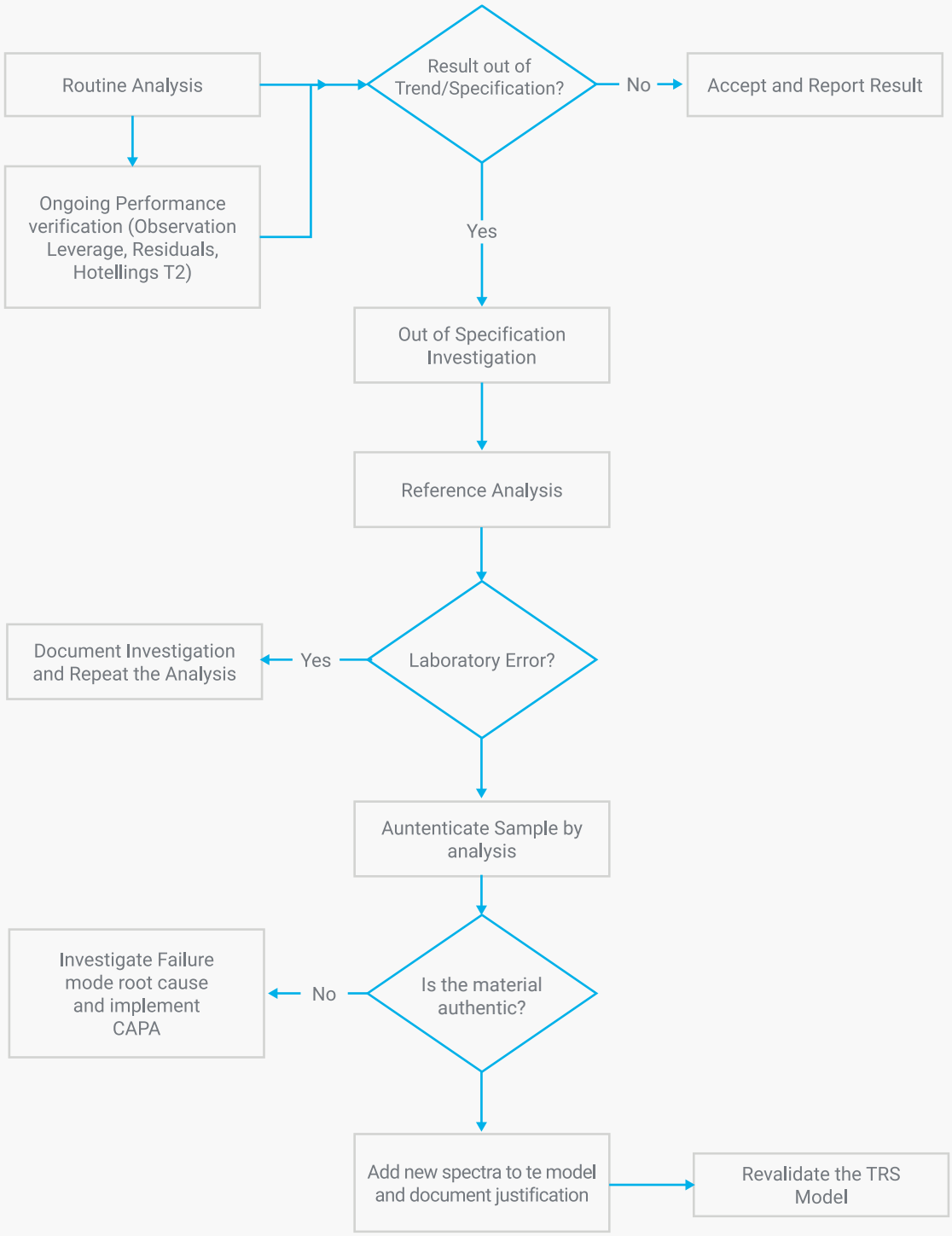


Figure 30. Period reference comparison process.

7. Method submission

At the time of writing (2018), there is a lack of Raman-specific guidelines from the European Medicines Agency (EMA) or the United States Food & Drug Administration (FDA). This is a challenge to users adopting Raman technology because there is no agreed 'road map' from the Competent Authorities (CAs) to follow. The absence of specific guidance can also be a challenge for the Regulatory Assessors. Due to the lack of prior experience, regulators fall back to sound scientific principles, such as the applicant must demonstrate the robustness of the technique and the rigor of the validation approach.

Fortunately, the guidelines on the use of Near Infrared Spectroscopy (NIRS) by the pharmaceutical industry and the data requirements for new submissions and variations, EMEA/CHMP/CVMP/QWP/17760/2009 Rev2 Jan 2012, became effective in August 2014. [14,16]

Due to the similarity in method development and chemometric approaches between NIR and Raman technology, the CAs recommend that this guidance is referenced, and that the requirements are applied for the introduction of Raman spectroscopy.

There are also some compendial guidelines available that offer guidance on the analytical considerations for Raman spectroscopy:

- European Pharmacopeia 2.2.48 [17]
- United States Pharmacopeia <858> and <1858> Raman Spectroscopy [18]
- United States Pharmacopeia <1039> Chemometrics [9]
- ASTM E1840-96(2014) [19]

This section focuses on the European process; however, as explained in Section 7.1, other regulatory authorities have similar processes and guidance. See references in section 10 for other guidance documents.

7.1 What is the method submission process?

Applying innovative technology for pharmaceutical analysis can seem a daunting task with many perceived obstacles, real and imagined, ready to get in the way of rolling out new technology. That said, we hope to have demonstrate, through this guide, that submitting transmission Raman spectroscopy methods to CAs is not so different from submitting any other new analytical chemistry method, that the requirements to meet are well understood, and help is at hand to support you in delivering a better way to rapidly test your products.

Problem statement:

- I want to change from using a standard HPLC method to transmission Raman spectroscopy.
- What type of variation do I submit?
- What documentation is required by the regulators?

The first step should always be to engage the regulators/National CAs as early in the process as possible.

For European updates, it is necessary to submit a variation to the relevant competent authority.

A variation is the procedure for submitting changes in the registered information provided to the Regulatory Authorities.

In the EU, Regulation 1234/2008 governs the procedure for the variation of marketing authorizations (licenses) and details the variation classifications:

- Type IA: Minor variation: Very little impact on the Quality, Safety and Efficacy of the product. Do and tell change.
- Type IAIN: Minor variation, the change must be notified within two weeks of implementation.
- Type IB: Minor variation, the changes must be notified to the Competent Authority before implementation.
- Type II: Major variation, may have an impact on the Quality, Safety and Efficacy of the product and approval must be granted from the CA before implementation of the changes.

The correct classification of the change is crucial to the applicant for right first-time assessment.

In the example given (changing from HPLC to Raman spectroscopy), the potential for an impact on the Quality, Safety, and Efficacy of the product must be assessed and therefore the change defaults to a type II variation. However, it is recommended that the variation classification is discussed with the individual national competent authority before any variation submission. Type IB and type II have been used previously for transmission Raman submissions in Europe.

In the case of a type II variation, there are no defined documentation or conditions to be fulfilled in accordance with the variation classification guideline. However, the requirements are like those listed for the type IB variation (B.II.d.2 d) for a change in the test procedure for the finished product:

- Amendment of the relevant sections of the dossier, including a description of the analytical methodology, a summary of validation data, revised specification for impurities (if applicable).
- Comparative validation results or, if justified, comparative analysis results showing that the current test and the proposed one are equivalent. This requirement is not applicable in case of a new test procedure.

In addition to these requirements, a risk assessment should be carried out before the submission preparation to assess the risks that may adversely affect the performance of the procedure in delivering valid results. The submission must also include an updated expert report, in this case an updated QOS must be provided.

In the U.S., although different naming is applied for the changes, the process and documentation requirements are quite similar:

- PAS – Prior Approval Supplement, major change
- CBe0 – Changes being effective on day 0, minor change
- CBe30 – Changes being effective day 30, minor change
- Annual Report, minor changes

The change from HPLC to Raman spectroscopy would be submitted as a PAS – Prior Approval Supplement (major change).

7.2 What does a method submission look like?

The submission will include all documents updated as part of the change in the specific dossier sections:

Module 1

- 1.4.1 Quality

Module 2

- 2.3 Quality Overall Summary
  - 2.3.S Drug Substance (if the change is applicable to the drug substance testing)
  - 2.3.P Drug Product (if the change is applicable to the drug product testing)

Module 3 Drug Substance (if the change is applicable to the drug substance testing)

- 3.2. S.4. Control of Drug Substance
- 3.2.S.4.1 – Specifications
- 3.2.S.4.2 – Analytical Procedures
- 3.2.S.4.3 – Validation of Analytical Procedures
- Module 3 Drug Product (if the change is applicable to the drug product testing)
- 3.2.P.2 Pharmaceutical Development
- 3.2.P.5 Control of Drug Product
- 3.2.P.5.1 – Specifications
- 3.2.P.5.2 – Analytical Procedures
- 3.2.P.5.3 – Validation of Analytical Procedures

7.3 Considerations

The content of each document submitted should be clear and unambiguous.

To aid right first-time submission, a list of key considerations for the crucial documents has been included:

Analytical procedure

- The scope of the procedure should be clearly described in the method.
- All details included must be specific to the product in question.
- Tables should be used to present key data, for example, descriptions of the detector laser type and wavelengths used, software used and the chemometric principles behind it, instrument calibration, validation and routine measurement, preprocessing, number of scans to be performed on each sample, and more.
- The method should clearly describe any differences in instrument setup and sample preparation for each application, such as bulk assay, content uniformity.
- Correlation of Raman and the reference method data should be described clearly so that there is no ambiguity as to how the data are paired.
- A description and justification should be provided as to how outliers in the data are to be dealt with in routine analysis.
- It should be clearly stated that, once the Raman method is nominated for ID, assay, or CU for batch release of the product, it cannot be substituted by the current registered method in the event of a batch failure without a full investigation of the failure and determination of the cause of the failure.

Pharmaceutical development

- Development issues should be discussed briefly.
- Details of the composition of the drug product must be included.
- Full details of the formulation variants must be declared.
- Formulation ranges for the active substances and excipients should be listed.
- Population blends, batch compositions, and blend/batch codes should be declared.

Analytical specification

- The specification should clearly state which identification, assay, and uniformity of content tests will be used routinely to batch release the product, for example, Raman or HPLC.
- The analytical tests for batch releases should be specified.

Validation of analytical procedures

- This section should fully describe how validation has been achieved for each parameter and the acceptance/rejection criteria applied to each parameter. The terms used should be unambiguously defined.
- All variables and parameters studied in the validation (such as the spectral acquisition of parameters and their optimization, external and internal validation, Raman and reference method pairing data, spectral quality test, the standard

error of prediction (SEP) and the acceptance/rejection criteria) should be fully explained.

– Circumstances under which the method will be revalidated, or the library will be updated, should be fully described.

Quality overall summary

– The QOS should be updated and should critically evaluate the Raman methodology or its validation for the intended use.

In general, all definitions should be described in detail and all terms should be obvious to the reader.

Periodic comparative analysis on the same sample using both Raman and HPLC testing is advised at least on an annual basis.

8. Authors

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9. Glossary

API	Active Pharmaceutical Ingredient
ASTM	American Society for Testing and Materials
AV	Acceptance value
CA	Competent Authority
CU	Content uniformity, aka. uniformity of content
DoE	Design of experiments
EMA	European Medicines Agency
EP	European Pharmacopeia
FDA	U.S. Food and Drug Administration
HPLC	High performance liquid chromatography
ICH	International Council for Harmonization
ID	Identification (usually of drug product
L-HPC	Low-substituted hydroxypropyl cellulose
MCC	Microcrystalline cellulose
NIR	Near infrared
NMT	Not more than
PAS	Prior Approval Supplement
QOS	Quality by design
RAM	Rapid analytical method
RMSE	Root mean square error
RSD	Relative standard deviation
SEP	Standard error of prediction
SNV	Standard normal variate
TRS	Transmission Raman Spectroscopy
UPS	United States Pharmacopeia
UV-vis	Ultraviolet-visible spectroscopy

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