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<DRAFT>

GUIDELINE ON THE USE OF NEAR INFRARED SPECTROSCOPY BY THE PHARMACEUTICAL INDUSTRY AND THE DATA REQUIREMENTS FOR NEW SUBMISSIONS AND VARIATIONS

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Comments should be provided using this template to qwp@emea.europa.eu

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GUIDELINE ON THE USE OF NEAR INFRARED SPECTROSCOPY BY THE PHARMACEUTICAL INDUSTRY AND THE DATA REQUIREMENTS FOR NEW SUBMISSIONS AND VARIATIONS

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EXECUTIVE SUMMARY

Near Infrared Spectroscopy (NIRS) has become a well established technique and has been used for several years in the pharmaceutical industry. The technique is useful for the identification and assay of pharmaceutical starting materials, intermediates and finished products, as well as for in-process control and monitoring purposes. NIRS constitutes one of the major methods in Process Analytical Technologies (PAT).

This guideline provides guidance on the development, calibration, validation and maintenance of NIRS methods and the data to be submitted to the competent authorities when NIRS is the subject or part of a marketing authorisation application.

This guideline also clarifies and differentiates the data requirements for the marketing authorisation dossier and those for GMP, including change control.

This guideline should be read in conjunction Annex I to Directive 2001/83 (medicinal products for human use) and Directive 2001/82 (medicinal products for veterinary use) as amended, other EMEA documents and the European Pharmacopoeia, especially:

- Ph. Eur. Monograph 2.2.40.
- ICH Note for Guidance on Validation of Analytical Procedures CPMP/ICH/381/95 and VICH Guidelines GL1 & GL2 on Validation of Analytical Procedures CVMP/VICH/590/98 & CVMP/VICH/591/98
- Note for Guidance on Process Validation CPMP/QWP/848/96 & EMEA/CVMP/598/99
- ICH Q8: Pharmaceutical Development
- ICH Q9 Quality Risk Management
- ICH Q10 Quality System

1. INTRODUCTION (background)

1.1 Regulatory Status

Normally a NIRS method is used as an alternate method to one or more validated conventional methods specified in the quality part of the dossier (the ‘reference methods’).

As a NIRS method generally needs to be developed and validated in conjunction with these reference methods and cannot be repeated easily by official control laboratories, these reference methods and corresponding specifications should remain in the authorised specifications, even for applications for which NIRS is an element of PAT.

Once the NIRS method has been approved by the competent authorities, the specification ‘if-tested’ may be stated beside the reference method.

1.2 Characteristics of NIRS

The main stages in developing and establishing NIRS methods are summarised as follows:

- Development

  Scientific Basis and scope, Apparatus, Sample Preparation, Elements Affecting Spectral Response, Instrument Performance, Feasibility Study

- Data collection

  Pre-treatment of data, Preparation of representative samples, Calibration and Validation sets, Spectral library, Reference methods

- Calibration

Software chemometric modelling, Optimisation, Avoidance of under and over fitting

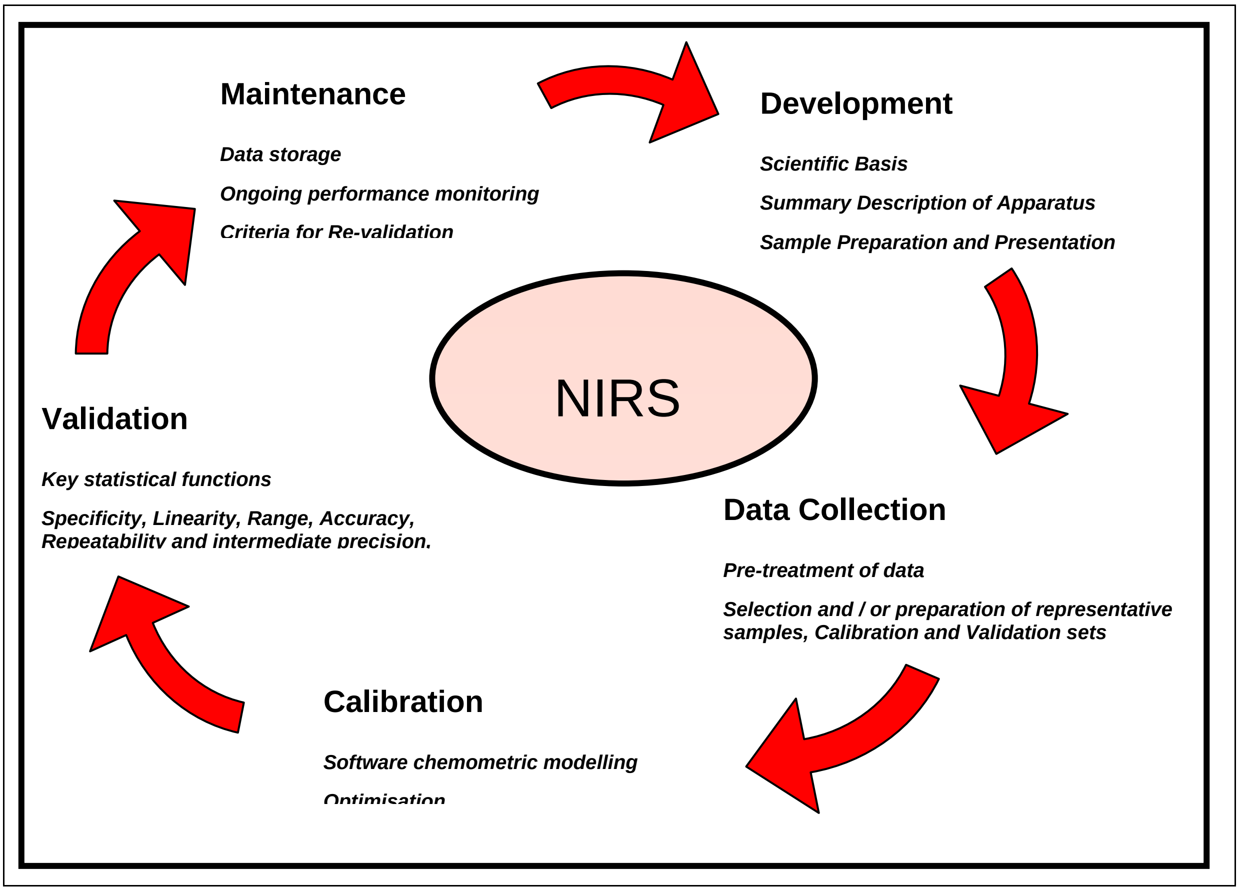
- Validation

  Key statistical functions, Specificity, Linearity, Range, Accuracy, Precision, Robustness,
- Maintenance

  Data storage, Performance monitoring, Re-validation, Change management, Method Transfer

It is recognised that the development and implementation of a NIRS method is iterative and that the stages are interdependent (see Figure 1).

Figure 1 The Iterative nature of NIRS



With the statistical capability of much of the current NIRS software, it is possible to develop a NIRS method with minimal understanding of the relevant chemometrics, with the consequential high risk of invalid results arising from the influence of unknown hidden variables.

It is therefore emphasised that the training and skills of the NIRS analysts responsible for developing the method are of critical importance in providing assurance that the method is fit for purpose.

The understanding, experience and expertise of the NIRS analysts should be apparent from the quality of the narrative and data submitted with an application. This should be supported by an appropriate CV, Qualification and practical experience of the personnel involved in the use of NIRS should be subject to particular attention during GMP inspection.

For NIRS methods, it is possible to subject the calibration model to continuous revision and refinement as new data becomes available following the purchase or production of new analyte batches.

It is consequently possible to extend the calibration model continuously. This is considered good practice for such iterative methods and is recommended. In support of this, once the calibration model and change protocols have been approved, then subsequent changes of the NIRS method, to extend the calibration model, need not necessarily be subject to variation. Satisfactory management of these changes does however fall within the remit of GMP and the data supporting these changes should be available for inspection (see Section 8 on variations). The change control procedure should address the extend of calibration model.

2. SCOPE

NIRS differs from conventional analytical techniques such as HPLC or GC because chemometric techniques are required for interpretation of the analyte signal. NIRS has been described in the European Pharmacopoeia since 1997 and adopted in 2005, however a single reference to the Monograph is considered insufficient for registration or variation.

NIRS is used for qualitative and quantitative analysis. It may also be used as a process analytical technology (PAT) for monitoring and controlling drug substance synthesis and finished product manufacturing processes.

NIRS may be employed in different ways, with different requirements, for defined purposes:

- A NIRS method is referred to as a primary method if it is used for final release of a drug substance or a finished medicinal product. NIRS may be used at-line for Real-Time-Release (RTR) when the method becomes the release method. For RTR the existing regulations should be noted.
- A NIRS method is referred to as an alternative method if it is used as an alternative method to a conventional (reference) method (e.g. chromatography, identification tests).
- A NIRS method is referred to as an in-process control when the method is used as a part of control strategy. This may be a part of RTR-testing. Further differentiation may be required in future (i.e. NIR for RTR, NIR for IPC)
- A NIRS method may also be used for data-mining purposes (process knowledge), if the method is linked to elements of a PAT concept. In this case, NIRS is only used to enhance process understanding

This guideline describes the data to be provided in the dossier for a marketing authorisation or a variation and clarifies which data should be considered part of GMP.

This guideline does not address the use of NIRS method for containerwise confirmation of identity of in coming starting materials supplementary with the use of primary method connected with an appropriate statistical representative composite sample. This particular use is relevant only to GMP practices.

3. LEGAL BASIS

This guideline supports applications that include NIRS methods for marketing authorisations according to Directive 2001/82/EC, as amended and Directive 2001/83/EC, as amended and for variations submitted according to relevant legislation in place at the time.

4. GENERAL REQUIREMENTS

4.1 Development

4.1.1 Scientific Basis and Establishing the Scope of the method

NIRS has a wide range of quantitative and qualitative applications e.g. assay of drug substances and excipients, moisture content, particle size, tablet hardness, identification and reaction or process monitoring. However, the limitations of the method, namely in sensitivity and selectivity, mean that its application requires a sound understanding of the physico-chemical basis on which its measurements rely and of the instrumental and chemometric principles involved.

The broad responsiveness of NIRS to the potentially many chemical and physical attributes of the sample matrix under examination, with its relatively weak and overlapping spectral bands, can be confounding and make it difficult to determine if the specific attribute under consideration is differentiated by the method. The applicant should discuss the scope and purpose of the NIRS method under development and show it to be unambiguous, appropriately limited and justified, (see also 4.6) supported by spectral evidence that the NIRS response is relevant to the analyte or property under consideration e.g. evidence that the drug substance exhibits a characteristic NIR absorption.

The samples used for model development should be relevant to and within the scope of the purpose of the method.

The NIRS method should, as a pre-condition, be able to reject samples that are outside of its defined scope (e.g. out of range, compositionally incorrect).

Following on from this, the complex informative content of the NIRS signal requires chemometric modelling, using sophisticated statistical software packages. These work by correlating, in a purely empirical fashion, the variance in the NIRS signals to a number of latent variables or factors, constrained by a set of calibration reference data. There is always a risk that the correlations identified by the software are due to chance only and not to changes in the analyte. To minimise this risk, the software must be used in a systematic way to prevent the chemometric model from becoming divorced from the physics and chemistry that forms the basis of the analysis.

Signal pre-treatments may be used to eliminate or minimise those components of the NIRS signal that can be shown not to include the analyte signal and are therefore irrelevant. This minimises the potential for chance correlations. Pre-treatments are an important element in method development.

It is important that the applicant identify assumptions made during development, which should be described and justified. For example, an assumption of linearity of the NIRS signal to changes in the analyte or property under consideration would be acceptable, on fundamental spectroscopic grounds, for drug substance assay, but should be proven for other attributes e.g. particle size, hardness.

The strengths and weaknesses of the proposed method should be discussed and these taken into account in calibration and validation.

4.1.2 Summary Description of Apparatus

Summary pertinent details of the apparatus should be provided, including the instrument manufacturer and model number, the instrument type (e.g. filter, grating, FT, acousto-optic tuneable filter (AOTF), diode array), spectral range, resolution, sampling devices, and any other additional components or controls considered necessary for the proposed method.

The measurement method e.g. transmission, diffuse reflectance, transflectance, should be described.

The means of data collection and analysis, and associated software packages should also be described.

The suitability of the apparatus and software for the intended use should be discussed, including software validation.

The statistical parameters used should be unambiguously defined and their function fully described. These should be consistent with those described in the glossary.

4.1.3 Sample Preparation and Presentation

A ‘sample’ in this guideline refers to an individual batch and should not be taken to mean samples from a single batch (composite sample).

Details of sample preparation, if any, should be provided, justified and shown to be robust.

The means by which the sample is presented to the NIRS detector should be described. The impact of possible variations in the presentation on the NIR response should be discussed, supported by appropriate data, and, if shown to be significant, demonstrated to be satisfactorily controlled.

The spectral range employed should be described and justified.

The number of scans recorded per sample should be stated and also justified.

4.1.4 Elements affecting Spectral Response

Background physical and chemical elements may be present, both internal and external to the sample, possibly uncontrolled and outside the scope of the proposed NIRS method. These could influence the spectral response obtained, introducing variance and bias and undermining calibration and validation.

It is not possible to list all possible interfering elements, but these include: the environment in which measurement takes place; sample temperature; residual moisture and solvents; sample thickness; sample optical properties; optical quality of the glassware; polymorphism; particle size; homogeneity and the age of the samples. Time of measurement and instrumental drift should also be considered.

The relevance of the interference of any element is dependent on the nature of the method, but may be wider in extent when little or no sample preparation is involved.

Each potential interfering element that may affect the spectral response should be considered and discussed in turn and either shown to be insignificant or satisfactorily controlled, supported by appropriate data.

4.1.5 Verification of Instrument Performance

It should be confirmed that the instrument is verified according to manufacturer’s recommendations and that the instrument complies with the requirements of the Ph. Eur. 2.2.40 Near-Infrared Spectroscopy. Qualification protocols, reports and periodicity of re-qualification should be subject to GMP inspection.

4.1.6 Feasibility Study

A feasibility study should be undertaken to show that NIRS analysis is possible, to include for example, evidence that a suitable NIR response can be obtained, linearity, investigation of potential confounding factors, matrix interference, sample handling and preparation, as discussed above.

The capability to reject out-of-scope samples and identify outliers should also be investigated.

A report of the feasibility study should be provided

4.2 Data Collection

4.2.1 Selection and/or preparation of representative samples, calibration or validation sets

Sample collection and population

Samples for NIRS analysis should be representative of routine production and should therefore be collected according to approved standard operating procedures for sampling and reflect the established manufacturing process capability.

Before any NIRS measurement takes place, it is very important to optimise the presentation of the sample to the NIRS instrument. Examples of factors that should be optimised are sample orientation, sample size, optical quality of glassware and environmental conditions.

The sample population for a quantitative or a qualitative method should cover all potential variation that may be encountered in routine production. Such variation may include:

- Concentration of the analyte in question
- Particle size
- Suppliers
- Water content
- Residual solvent content
- Variations in the matrix (excipients)
- Process variation (samples collected over an extended period)
- Sample age

Outliers

Any suspected 'out-of specification' results from the calibration and validation sample sets should be re-analysed using the reference method. The exclusion of any such samples should be documented and justified by the applicant.

If a sample is rejected and shown to be an outlier because of characteristic properties, the rejected sample should be verified using an appropriate alternative analysis. After the confirmation of authenticity, the sample should be included in the spectral reference library and the model should be fully re-validated so as to include this source of variation.

Procedure for handling of "out-of specification" results should address outliers and recording should be subject to GMP inspection.

4.2.2 Pre-treatment of Data

Given that NIR spectra are affected by physical parameters such as particle size and sample presentation, raw NIR spectra are mathematically manipulated prior to development and testing of the calibration model. Such manipulations may include smoothing, baseline correction or derivatisation, which are performed in order to remove unwanted sources of variation from the data prior to manipulation and to enhance spectral features.

NIRS signal pre-treatments are used to reduce the number of latent variables or factors (see Section 6.3.3) and consequently sample numbers.

Caution must be exercised when performing any pre-treatments because artefacts can be introduced or essential information can be lost. An understanding of the algorithm is required and in all cases the rationale for the use of pre-treatments should be documented.

Serial pre-treatments such as normalisation followed by derivatisation should be specified; reference to ‘standard pre-treatments’ is unacceptable. Any pre-processing of the data carried out by the software by default should be stated. Given that calibration models are generated based upon the variation present in the spectra, the selection of any additional pre-treatment should be justified. Exemplary spectra to demonstrate the effect of the pre-treatments should be provided and discussed.

4.2.3 Establishment of a Spectral Reference Library

The composition of the spectral reference library should cover the scope of the NIRS method and should be subject to a change control process subject to GMP inspection.

Batches should be representative of the marketed materials or products and laid down in a list of batch numbers.

It should be verified that the spectra used to create the spectral reference library are correctly recorded according to the defined analytical procedure.

For qualitative analysis, where the spectral reference library is very large or diverse, it may be useful to divide the library into appropriate ‘sub-libraries’ to avoid calibration models becoming too complex. The choice of subsets and the degree of sub-libraries should be described and justified.

4.2.4 Analysis by the reference method

The samples used for NIR calibration and validation require quantitative values to be assigned for the attribute under consideration or authentication for the attribute under consideration and therefore require analysis by a suitable reference method.

Ideally, reference measurements should take place at around the same time as NIR scanning. Any differences between sample treatments in the reference and NIRS methods should be stated (e.g. grinding or blending).

The suitability of the reference method should be justified with respect to the intended purpose of the NIRS method.

Data to support the choice of reference method should be provided and should include:

- A description of the analytical procedure according to Module 3.2.P.5.2[1] data requirements.
- Details of the validation of the analytical procedure according to Module 3.2.P.5.3[1] data requirements and ICH Q2(R1) Note for Guidance on Validation of Analytical Procedures: Text and Methodology (CPMP/ICH/381/95) (for Veterinary applications: VICH GL1 & GL2 Validation of Analytical Procedures CVMP/VICH/590/98 & CVMP/VICH/591/98).
- Details of relevant reference standards and materials according to Module 3.2.P.6[1] data requirements.

4.3 Calibration

Specific requirements for calibration are described in the sub-chapters for ‘Quantitative Methods’ and ‘Qualitative Methods’.

4.4 Validation

Validation of NIRS methods should comply with the guidance given in ICH Q2(R1) Note for guidance on validation of analytical procedures: text and methodology (CPMP/ICH/381/95) (for

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[1] Or equivalent in the Notice To Applicants format for Veterinary dossiers.

Veterinary applications: VICH GL1 & GL2 Validation of Analytical Procedures CVMP/VICH/590/98 & CVMP/VICH/591/98) and data requirements for Module 3.2.P.5.3[2].

The validation set of samples should be completely independent of the calibration set (see 5.4 and 6.4). The suitability of the validation set, including its independence from the calibration set, should be discussed and justified.

The basis of the validation is the comparison of results obtained by analysis of the same set of samples by the NIRS and reference methods.

In all cases, the acceptance criteria for validation should be specified and justified with reference to the intended purpose of the method.

If the NIRS method is being presented in the initial registration dossier, validation data should also be presented for the reference analytical method (see section 4.2.4 Analysis by the reference method).

If the NIRS method is being registered as a variation to a licence in which the reference method is already approved, then a summary of the validation data for the reference method, in compliance with CPMP/ICH/381/95 (or CVMP/VICH/590/98 & CVMP/VICH/591/98), should be provided.

Departure from guidance should be justified and will be considered on a case-by-case basis.

4.5 Change Control and Maintenance

4.5.1 Planned and Unplanned Changes

Changes (both planned and unplanned), that might affect the performance of a NIRS method may necessitate re-validation of the whole NIRS model to demonstrate continued selectivity and robustness.

Change control protocols are important for qualitative methods where spectra of batches are likely to be added to the spectral reference library to keep it up to date and to all NIRS methods in case of relevant changes to the instrumentation, which cannot be controlled by the analysis of performance verifications alone (e. g. changes in the physical properties of the substance or in the source of supply).

A relevant change control protocol, in compliance with EU GMP requirements Annexes 15 and 20, should be submitted. The implementation of this protocol will be subject to GMP inspection.

Changes should be fully documented, and include appropriate re-validation and comparability reports, to show that the revised method is consistent with that approved. When appropriate the dossier should also updated by variation. (see Section 8)

For qualitative methods, suitable change control tests should be in place for each method and spectral reference library. This test should be composed of a minimum of two standard sets (i.e. two classes or substances) for which separation is most critical. If the NIRS method does not comply with the change control test, it should be fully re-validated. It should be demonstrated that the suitability of the change control test remains stable over time.

Change control requirements specific to quantitative methods are included under Section 8.

Some potential changes, including changes to instrumentation, can be included in the calibration model. Data generated to demonstrate robustness may show that some changes have no effect.

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[2] Or equivalent in the Notice To Applicants format for Veterinary dossiers.

Comparison of the chemometric results applied on the spectra present in the spectral reference library with the current and replacement software is suitable as change control test but should still be described, including details of any statistical analysis performed.

4.5.2 Revalidation

The NIRS method should be challenged periodically with the related reference method to ensure its ongoing validity. A sample of a batch should be analysed by both the reference method and the NIRS method and the results compared. For quantitative methods, revalidation should be carried out at minimum, centre and maximum concentrations in calibration range.

Requirements for revalidation should be discussed and justified in the change control protocol. Revalidation reports and relevant recording should be subject to GMP inspection.

4.5.3 Out of Specification results in routine batch analysis

An out of specification result for routine batch analysis by the NIRS method should result in rejection of the batch.

If, on investigation, the batch complies with the specification using the reference method, then this may indicate that the NIRS method is not been fully optimised (e.g. there may be over-fitting (see 6.3) and the out of specification result is a false negative.)

Alternatively, the inconsistency in results between the NIRS and reference method may highlight a more fundamental issue in the characterisation of the quality of the product.

The affected batch should not be released onto the market, without investigation and justification.

For quantitative analysis, if it is confirmed that the NIRS model was not optimal, then the method should be redeveloped appropriately. In these circumstances, the change in the NIRS method should subject of regulatory review, by variation.

If the batch on re-analysis using the redeveloped method is found to be within specification, and the variation is approved, then they may be released onto the market.

For qualitative analysis, an appropriate change control report, including details of the results and conclusions of the investigation and any necessary revalidation, should be prepared and made available for GMP inspection.

4.5.4 Extrapolation beyond the scope of the NIRS method

NIRS is valid only if interpolated within the defined scope of the method.

The use of a calibration model to analyse samples with characteristics outside of the defined scope of the method is not valid and would be considered a major GMP deficiency.

The extension of the scope of the method (e.g. widening of the range of interest) should by variation only.

4.6 Summary of Data Requirements

The following should be provided:

- The scope of the method

  The aims of the method should be specified, appropriately limited and justified and include details of instrumentation and software, sample preparation and handling, spectral pre-treatments, factors affecting spectral response, and verification of instrument performance

- A report of the feasibility study

- Details of the composition of the calibration and validation sets, with justification
- Description of the reference method
- Details of the establishment of the spectral library
- Calibration report
- Validation report
- Change Control Protocol(s)

5. QUALITATIVE METHODS

5.1 Development

NIRS has a wide range of qualitative applications, almost all of which could be divided into three major areas:

- Identification
- Qualification
- Conformity Checks

In Pharmacopoeial monographs, identification is defined as the confirmation of a certain chemical entity. However, the pharmaceutical industry uses a wider concept, implying that identification may also include differentiation between different qualities of one chemical entity (e.g. particle size, polymorphs).

To allow differentiation, this guideline uses the terms **identification** (only chemical structure) and **qualification** (chemical- and physical attributes).

Conventional identification is generally based on more than one analytical method. Consequently, it should be clear, if applicable, which reference methods will be replaced by the NIRS method. Tests used for identification or qualification are those performed for inspection of incoming raw materials, intermediates or finished products.

In addition to the definitions for identification and qualification, this Note for Guidance uses the term **conformity** as the conformation of characteristics in accordance with a certain degree of similarity (chemical and/or physical attributes) to a specified standard (e.g. standardised spectra). Conformity checks are often used in manufacturing processes as in-process controls for monitoring purposes or as a part of a PAT-concept (e.g. determination of an endpoint).

The identification or qualification of a substance (e.g. drug substance, excipient, blend, drug product, intermediate) using NIRS is based on the comparison of the spectral data of the substance with the spectral data of several samples of several batches of different substances present in a spectral reference library. Chemometrics will usually be necessary to compare the data and to draw conclusions (pass, no match or ambiguous). The appropriate confidence level of the conclusion should be defined statistically and justified.

If an ambiguous conclusion is obtained, the method should be adjusted such that the substance will be correctly approved or rejected, or those substances that interfere should be excluded from the scope of the method. Interfering substances or grades of substances may also be classified as one single entity if possible (e.g. different grades of lactose).

The classification of a substance can be done in several stages. For example, a classification of chemical identity or a group of related substances may be performed, followed by application of more selective models for each individual grade or substance. This approach can be used to decrease the likelihood of false positives/negatives. Qualification is often performed after the identification of the sample has been ascertained. In this case, a model for qualification measures how well a sample fits in with a model which is derived from samples chosen to represent the defined variability of a chemically identical substance.

Sample preparation, presentation and the consideration of elements affecting the spectral response should be carried out as described in the Section 4.1.

A feasibility study should be performed and reported as discussed in Section 4.1.6, which should show that separation of relevant materials/substances is possible.

5.2 Data Collection (qualitative methods)

5.2.1 Selection and/or preparation of representative samples, calibration and validation sets

Sample collection and population

The selection of samples, and where necessary the subsequent extent of spectral library development, will depend on the complexity of the application. All samples should be verified with the conventional reference methods, which are included in the registered specification. The validation of the method should demonstrate that spectra of an acceptable minimum number of batches have been included in the calibration and validation sets and that these batches are sufficiently representative to cover the normal variation of the substance.

All samples for qualitative methods should be representative of routine practice and should therefore be collected according to standard operating procedures and in line with established manufacturing process capability. The sample population should cover all potential variation that may be encountered routinely, e.g. particle size, suppliers, moisture, residual solvent content, variations in the matrix (excipients) and process variation (including samples collected over an extended period).

Where laboratory or pilot scale samples are required to expand the narrow range of production samples to properly assess sensitivity in line with specification limits, such samples should be prepared using the same manufacturing procedure as used for routine batches.

The balance of production to development batches in all sample sets should be justified with respect to the variation expected in routine production. The choice of samples should be sufficient to ensure the robustness of the method for routine use.

For conformity check (in-process controls and monitoring purposes) the reliability of the chosen method should be demonstrated by appropriate validation according to Section 4.4. Depending on test type, the validation may be less extensive, if justified.

Number of samples

The number of samples to be included in the spectral library in order to create a valid calibration model for qualitative analysis will depend on the complexity of the sample matrix and/or NIR signal and should be fully justified. In general, the more complex the sample matrix, the more samples will be required to cover the statistical population. A minimum of three or more spectra of *at least* six batches (together called the training set) are required. The number of batches should be sufficient to cover normal production variation and should be justified.

Composition of training set, training test set and validation sets

In order to develop, optimise and validate a calibration model for a qualitative method, the following sets of samples are required:

- The training set for creating the calibration model
- The training test set for optimisation and choice of the calibration model (if necessary)

- The independent validation set for validation of the proposed chosen model

Each set of samples should be representative of the scope of the NIRS method, as defined under section 6.2.1, and include samples covering the full range of potential variation in the sample population (both qualitatively positive and negative).

The selection of the appropriate calibration model may be supplemented either by so-called ‘internal validation’ or ‘calibration test set validation’ methods. ‘Internal validation’ is the application of resampling statistics such as cross-validation or bootstrapping. In summary, subsets of the spectral reference library data are subjected to a variety of statistical processes to identify which calibration model may best fit the available data.

‘Calibration test set validation’ is the application of the variety of calibration models generated using the training set of samples to the training *test* set (for which spectral and reference method data are available), which are drawn from the same population as the training set, but are not used to generate the calibration model. The descriptive statistics for the training test set are used to determine whether the model is optimal.

The chosen model should differentiate all samples and verify them unambiguously.

Outliers

Only in exceptional cases may outliers appear in a qualitative spectral reference library. Therefore, the exclusion of such a sample should be carefully justified by the applicant (see also Section 4.2.1, subsection Sample collection and population).

5.2.2 Analysis by the reference method

For sample qualification, the additional information for further differentiation of a substance according to other characteristics such as grade, polymorphic or hydrate form should also be carefully ascertained by suitable reference methods (see Section 4.2.4).

5.3 Selection of calibration model

The selection of the most appropriate method of calibration depends on the scope of the spectral library. In general, the simplest available model which gives successful results should be used.

There are many different kinds of classification methods which could be divided into several categories. Common methods are Principal Component Analysis (PCA), Discriminant Analysis (linear or quadratic), Soft Independent Modelling of Class Analogues (SIMCA), Cluster Analysis (dendrograms), k-Nearest-Neighbourhood-Analysis (kNN-Analysis) and Supported Vector Machines (SVM).

All of these methods deal with the measurement of distances in multidimensional space between an unknown sample and a well-defined group of samples or additionally within this group of samples (e. g. Euclidean Distance, Mahalanobis Distance/Leverage).

It is almost always necessary to determine thresholds or/and confidence limits for a proper verification of samples. To optimise the performance of the chosen method, characteristic performance values should be in the validation procedure.

Optimisation

In general, the optimisation of a qualitative method is confined to the selection of the samples included in the spectral reference library and the chosen calibration model.

5.4 Validation

Validation of a qualitative NIRS method should consist of validation for specificity (selectivity) and robustness. Possible adjustments are a change of pre-treatment and a change of thresholds, expulsion of substances from the scope of the method or classification of substances as one. The results of the final validation should be submitted to the competent authorities with a summary discussion.

The objective of an internal validation is to ensure the performance of the spectral reference library. Generally this is evaluated by testing the samples of the spectral reference library using cross-validation techniques or a where necessary a discrete training test set. This step should demonstrate that all samples of the spectral reference library are verified unambiguously by the chosen model with defined thresholds or/and confidence limits.

External validation of the method should demonstrate the performance of the chosen model by an independent validation set consisting of samples which were not used in the creation of the spectral reference library.

Unlike other qualitative methods that could be used to identify or verify defined qualities, NIRS is not able to specify uniform statistical parameters of performance for identification or qualification.

However, the statistical parameters used to evaluate the performance of the model should be fully described and their suitability for the intended purpose should be justified.

As a general rule, using appropriate thresholds or confidence limits, it should be demonstrated that a confidence level of at least 0.95 is assured. Methods with a confidence level less than 0.90, are not considered acceptable.

The use of methods with a confidence level between 0.90 and 0.95 should only be accepted in exceptional cases and should be justified in detail (e.g. small number of characteristic samples at the beginning of method development). Where necessary a risk analysis should be presented by the applicant.

These thresholds are stated as a general rule only. Each model should be taken on a case-by-case basis. For example, if a confidence level of 0.99 is consistently achieved for the identification of a substance from a spectral library, then it would be expected that the threshold of acceptance would be tightened in line with the data obtained.

Specificity (selectivity)

The extent of specificity (selectivity) testing depends on the intended application of the NIRS method. Lack of specificity (selectivity) of the method may be compensated by other supporting analytical procedures.

Independent samples of substances represented in the spectral reference library, but not used to create it (i.e. different batches, blends), must be tested and all approved correctly (pass).

Potential challenges should be presented to the spectral reference library. These challenges should be rejected (no match). For the identification or qualification of pharmaceutical substances, relevant existing name- and structure-analogues should be included in the validation set, unless their absence is justified. Justification can be based on:

- The number of included analogues in view of the total number of existing analogues (the validation set should be sufficiently representative for the whole set of all existing analogues).

- The expected NIR spectral characteristics of the analogues.

- The probability of the presence of the analogues in the relevant pharmaceutical setting.

Where applicable (e.g. qualification applications), validation should include challenge with different grades of the same substance, anhydrous/hydrated material or different polymorphs etc. or material supplied by different vendors. Consideration should also be given to materials manufactured by external suppliers that could be delivered in error.

The results of the validation should demonstrate unequivocally that for each tested parameter, the NIRS method is sufficiently selective to discriminate between batches that comply with the tested parameter and batches that do not, in the same way as for the reference method.

The composition of the validation set should be described unambiguously and should be justified.

Robustness

Effects of possible, relevant variations e.g. temperature (environment and sample), humidity, different position of the sample in the optical window, different sample presentation devices, variation in sample bottles/vials, probe depth or, if applicable packaging materials, should be understood, tested and documented. Instrumental variations may also be considered in the validation for robustness, e.g. changing lamps, reflectance standard etc. As such, some variations and potential changes may already be included in the calibration during the development of the method.

The use of experimental design may be considered to maximise the information available.

6. QUANTITATIVE METHODS

6.1 Development

Feasibility Study

A feasibility study should be undertaken to show that quantitative analysis is possible (as discussed in Section 4.1.6) and should include evidence of linearity, as appropriate.

Examples of evidence of a suitable NIRS response include the submission of data showing that the net analyte NIR signal is significantly greater (>10) than the NIRS noise arising from the sample matrix, or using standard addition techniques, with appropriate spectral inspection.

The study should include an appraisal of the potential methods of calibration that could be used, estimation of the likely number of latent variables or factors and consequentially sample number requirements, together with clear definitions and descriptions of the proposed statistical parameters and indicators to be used for assessment of the method.

The capability to reject out-of–scope samples and identify outliers should also be investigated.

A report of the feasibility study should be provided

6.2 Data Collection

6.2.1 Selection and/or preparation of representative samples, calibration and validation sets

Sample collection and population

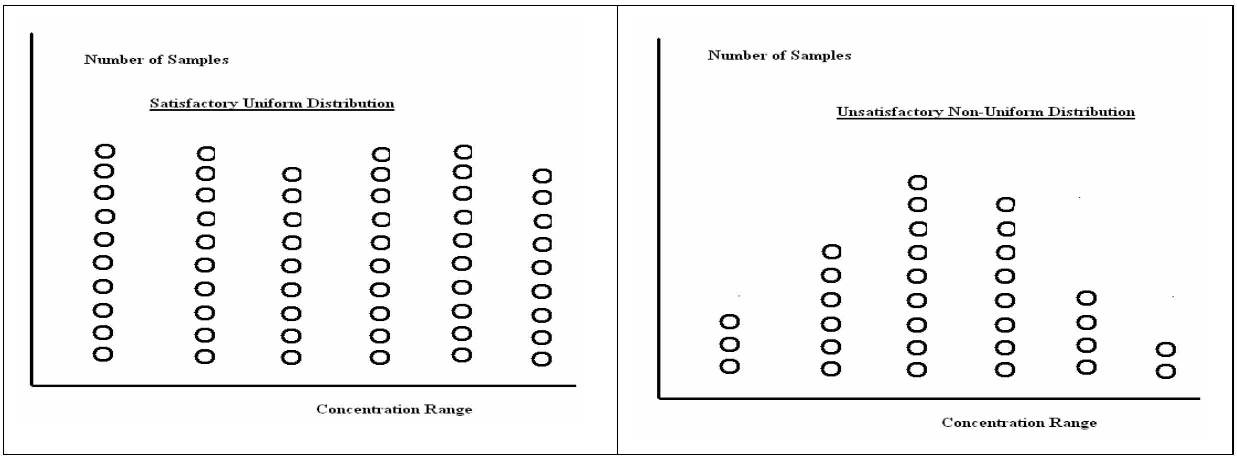
See Section 4.2.1.

Where feasible, samples of production batches should be augmented with those from development batches, manufactured specifically to simulate the limits of potential variation in the sample. Where laboratory samples are required to expand the narrow range of production samples to properly assess linearity in line with specification limits, such samples should be prepared using the same manufacturing procedure.

The balance of production to development batches in the sample set should be justified with respect to the variation expected in routine production.

In keeping with the fundamental assumptions made in the application of regression correlation statistics and to prevent bias, a uniform distribution of samples throughout the range of potential variation should be ensured (see figures below); a factorial experimental design may be used to this end. Evidence of this should be provided.

The choice of samples should be sufficient to ensure the robustness of the method for routine use.



Number of samples

It is expected that interference of the matrix components will be examined during feasibility studies and an appropriate sample collection method and calibration model chosen, together with an estimate of the number of latent variables or factors.

To avoid bias, the number of samples used to develop the calibration model should be very much greater than the number of such factors. A minimum standard of a 6:1 ratio of sample measurements to factors is proposed by the ASTM[1]. Another commonly used informal rule of thumb is a 10:1 ratio.

The number of samples to be included in the spectral library in order to create a valid calibration model for quantitative analysis will depend on the complexity of the sample matrix and/or NIR signal. In general, the more complex the sample matrix, the more samples will be required. For example, if the sample matrix consists of two simple components only, the number of samples required will be lower than if a multi-component, complex system is to be analysed. For the latter, a more complex chemometric model may be required, for which a greater number of samples will be required to ensure its validity.

The number of samples included in the calibration and validation sample sets should be fully justified.

Composition of calibration and validation sample sets

To develop, optimise and validate the calibration model for quantitative analysis, three sets of samples are required (similar to those described for qualitative methods, however nomenclature may be different):

- The calibration set for creating the calibration models.
- The calibration test set for optimisation and choice of calibration model (if necessary*)
- The independent validation set for validation of the proposed chosen model.

The calibration set of samples is used to generate potential calibration models and as such, should include samples covering the full range of potential variation, within the defined scope of the method (see Section4.1.1).

The optimisation and choice of the calibration model is normally undertaken either by so-called 'internal validation' or by 'calibration test set validation' methods (see Section 6.3.2).

It should be emphasised that this does not represent independent validation of the NIRS method, which must be carried out using a third, entirely independent set of samples.

*****Optimisation by 'Internal validation' is a statistical resampling method that reworks the calibration set data, such that the calibration test set of samples is not required. A fuller discussion of 'internal validation' is given in Section 6.3.2. This is commonly used when fewer samples are available.

'Calibration test set validation' is the application of the calibration models generated using the calibration set of samples to the calibration *test* set, which contains samples drawn from the same population as the calibration set, but which were not used to generate the calibration model. In practice, the calibration set often consists of two thirds of the available sample population. The calibration test set is the remaining third. The applicant should give the rationale for the composition of calibration and calibration test sample sets and justify their suitability.

The validation set is an entirely independent third set of samples, which should cover the full range of variation in the sample population. The size and composition of batches included in the validation sample set should be discussed and its suitability justified. This set is used to validate the calibration model generated either by 'internal validation' or 'calibration test set validation' and is used to generate the statistical parameter, the Standard Error of Precision (SEP), which is an indicator of the validity and predictive ability of the calibration model.

6.2.2 Analysis by the reference method

See Section 4.2.4.
The performance of the quantitative NIRS method is dependent on the performance of the reference method. Poor precision and accuracy of the reference method will limit the performance of the NIRS method. The suitability of the reference method should be justified with respect to the intended purpose of the NIRS method. It is important that care is taken to ensure that errors in the reference method are kept as small as practically possible and that appropriate reference standards are employed.

Repeated sample analysis by the reference method should be discussed and reference data should be tabulated and presented graphically. The number of replicates to be averaged to provide reference data for the calibration model should be stated and justified with reference to the performance (precision and accuracy) of the reference and NIRS methods.

6.3 Calibration

6.3.1 Software

Following acquisition of spectral and reference method analytical data of the calibration set of samples, it is necessary to carefully pair and match this data together prior to any chemometric modelling.

Using the paired data, the chemometric calibration model should be developed using the specified software package. Such software empirically characterises and correlates the variation within the data to a number of latent variables or factors (also called components).

The software can generate a variety of chemometric models, if desired, with differing factors or components, based on the type of model, such as PLS (using factors) and PCR (using components). Sample pre-treatments and other parameters may differ between models. The capability and application of the software used should be fully discussed and explained.

6.3.2 Optimisation

From the chemometric data generated by the software, the selection of the optimum calibration model is a pivotal step in the development of the NIRS method. It is dependent on the judgement and experience of the analyst to make the right choice, taking into account the known spectral behaviour of the analyte and sample matrix, the proposed scope of the method, the iterative nature of NIRS method development and statistical evidence of fitness for purpose.

Optimisation of calibration models is performed by ‘internal validation’ or ‘calibration test set validation’ methods as described in Section 6.2.1. These methods are used to aid assessment of the suitability of the calibration model in its ability to predict the correct quantitative result and in its validation.

‘Internal validation’ is the application of resampling statistics such as cross-validation, bootstrapping and leverage correction. In summary, subsets of the calibration set data are subjected to a variety of statistical processes to identify which calibration model may best fit the available data.

It is accepted that the use of resampling statistics for this purpose is a rapidly developing field and that more appropriate statistical processes may be possible, particularly relating to assessment of under and over fitting.

Each calibration model is characterised by a statistical parameter. For internal (cross) validation methods of optimisation, the characteristic statistic is the ‘Standard Error of Cross Validation (SECV)’.

‘Calibration test set validation’ is the application of the variety of calibration models to a set of samples (for which spectral and reference method data are available), which are drawn from the same population as the calibration set, but not used to generate the calibration model (see Section 6.2.1). The characteristic statistic for this method of optimisation is the ‘Standard Error of Prediction (SEP)’.

6.3.3 Selection of Factors or Components

The number of factors or components to be used in the calibration model is of critical importance to avoid under and over fitting of the data.

The scope of the proposed NIRS method and suitability of the calibration samples to adequately represent the product to be marketed should be taken into account when selecting the number of factors or components for inclusion into the calibration model.

If the calibration model is over-fitted (too many factors or components used), then it may be over specific to the characteristics of the calibration samples, creating a high risk that out of specification results are obtained for production batches that are fit for marketing.

If the calibration model is under-fitted, then the model may not match the intended scope of the NIRS method, having reduced specificity and creating a high risk that results in compliance with the specification are obtained for production batches that are unfit for marketing.

The following should be considered when choosing the number of factors or components to use:

- Co-linearity
- Minimal contribution to the data variance arising from the net signal of the analyte of interest
- Contribution to the data variance arising not from the net analyte signal, but from other components of the sample matrix e.g. excipients or other characteristics.

The above list is not exhaustive. The analyst should take into account all relevant issues revealed by the feasibility study and the known nature of the analyte.

The rationale for the choice of the chemometric model and the number of factors or components selected for inclusion into the model should be fully discussed and justified. This should include discussion as to why the model may be considered optimal.

Once selected, the proposed calibration model should be characterised, by graphical and statistical means. The characteristic statistic being the ‘Standard Error of Calibration (SEC)’.

6.3.4 Summary of Data requirements

A summary of the discussion and data to support the calibration model may include:

Software

a) A description of the capability and application of the software used and key statistical parameters used in modelling.

Factors or Components

b) A graphical representation of the variance accounted for by each factor.

c) A graphical representation of factor wavelength loadings and their comparison with the net analyte signal or other relevant signals.

d) Discussion of the suitability of factors for inclusion into the calibration model.

Optimisation

e) Discussion of the means of optimisation used e.g. using statistical resampling of the same calibration set data or use of the calibration test set (see 6.2.1).

f) Plot of SEP (when calibration test set validation is used) or SECV (when internal validation is used), or equivalents, against factors or components, with discussion.

Model

g) Discussion the process for choosing the proposed model.

h) Graphical presentation of the calibration curve of NIRS quantitative results against reference method results.

The correlation coefficient, calibration equation, slope and intercept, together with an analysis of residuals, as indicators of linearity, should be discussed.

i) Determination of the SEC and discussion of its suitability, including comparative SEC data with the other rejected chemometric models, if relevant.

Once developed, the calibration model is subject to validation to assess its predictive ability e.g. determination and evaluation of the SEP using an independent validation set (see 6.2.1).

6.4 Validation

6.4.1 General

See also 4.4.

The independent validation set of samples may be supplemented by specially prepared samples to demonstrate linearity, range and specificity (selectivity[3]).

The validation set should be quantitatively characterised by the NIRS and reference methods.

Since NIRS analysis relies upon reference data obtained from a primary method or very rarely, samples of known composition in order to impart meaning to the sample spectroscopic data collected, an additional statistical acceptance criterion is used, as a measure of the method's ability to predict the correct quantitative result is required. This is the 'Standard Error of Prediction (SEP)', for the validation set of samples (not to be confused with the SEP for the calibration test set: see also section 6.3.2).

Validation of quantitative NIRS methods should be demonstrated for the following parameters.

6.4.2 Standard Error of Prediction (SEP)

The SEP should be determined for the validation set and its suitability discussed.

This is considered a pivotal statistical parameter.

An indication of satisfactory performance for quality control[2] is given by the following parameters derived from the SEP:

Range/SEP-ratio and the Ratio of performance deviation (RPD). If equal to or greater than 10 and 5 respectively, then further justification of the suitability of the NIRS method should not be necessary.

The SEP should also not be larger than $1.4 \times SEL_{ref}$, unless justified in view of the required accuracy of the test method.

6.4.3 Specificity (Selectivity)

The specificity (selectivity) of an NIRS method is dependent upon the intended purpose, scientific basis and scope (see sections 4.1.1 and 6.1).

For specificity (selectivity), the method should be able to reject samples that are outside of its defined scope, such as out of specification product, placebo, samples containing different quantitative composition of proposed excipients, and samples containing different active substance and excipients.

The protocol of determining and assuring specificity (selectivity) should be provided together with relevant data.

The following may be used to support evidence of specificity:

a) Reference to the feasibility study data demonstrating that the suitable NIR response is based on the known NIR characteristics of the analyte (see section 4.1.6).

b) Comparison of the wavelength loadings, for the factors used to develop the chemometric model, against the known NIR characteristics of the analyte.

c) Validation data to demonstrate accuracy and robustness.

6.4.4 Linearity (correlation with reference data)

To demonstrate linearity, it is required that the validation set samples are distributed across the specified range of interest. Otherwise, linearity cannot be adequately confirmed and validated.

The NIRS results should be compared with those of the reference method the correlation coefficient and analysis of residuals (indicators of linearity), should be discussed, and supported by graphical representation.

The applicant should justify the choice of statistics applied to determine linearity if these differ from those described.

6.4.5 Range

The range of analyte reference values used to generate the calibration model determines the range of use of the method.

The range should be confirmed by use of a suitable validation set which matches in extent the proposed range.

Validation set samples having analyte content outside of the calibration range should appear as outliers when tested by the NIRS method (see also specificity).

6.4.6 Accuracy

Accuracy should be established across the specified range of the NIRS method and be comparable with the reference method.

The SEP should comply with the requirements given in 6.4.2 above.

6.4.7 Precision

Precision should be comparable with the reference method.

The SEP should comply with the requirements given in 6.4.2 above.

The suitability of the determined precision should be fully discussed and justified, in the context of the analyte of interest.

Repeatability

Dependent upon the nature of how samples are presented to the NIRS instrument, repeatability should be demonstrated through the analysis of replicate measurements. Repeatability should be demonstrated across the range of sample variation (ideally at three levels).

Intermediate precision

Intermediate precision should be demonstrated by the statistical evaluation of repeatability determined by different analysts over different days.

6.4.8 Robustness

Generally, the reference methods used to generate the primary data for the NIRS methods measure chemical *or* physical properties of samples whereas the vibrational characteristics measured by NIR spectral analysis take into account both physical *and* chemical properties.

Evidence to demonstrate the robustness of the NIRS method should therefore cover chemical and physical variables, dependent upon the purpose of the method and the conditions employed for sampling. These variables may include temperature and humidity, sample temperature, sample handling and instrument changes and is discussed in Section 4.1.4 “Elements affecting the Spectral Response”.

Furthermore, robustness should be addressed within the scope of the NIRS method (see 4.6). When this is the case, then reference to data generated from the development and optimisation of the calibration model and the validation data described above would be considered sufficient to demonstrate robustness. Otherwise, the protocol of determining and assuring robustness should be provided together with relevant data.

6.4.9 Limits of detection and quantification

Limits of detection and quantification for the proposed NIRS method need only to be demonstrated when relevant and where the analyte is considered an impurity (e.g. water content).

These are not required for the determination of content of the drug substance.

7. MATTERS SUBJECT TO GMP

These include:

- Software validation
- Training records, expertise and CVs, of NIRS analysts
- Qualification protocol (according to Ph Eur 2.9.40 requirements), Qualification reports, periodicity of requalification.
- Planned preventative maintenance and trend analysis.
- Change control protocol and its application e.g. extension of the calibration model arising from receipt of new batches or manufacture of new batches, change of the spectral library.
- Change control reports.
- Out of specification results procedure and recording. For quantitative analysis, it should be confirmed that affected batches were rejected, except in cases of regulatory approval by variation.
- Maintenance and version control of spectral library
- SOPs, analytical methods, validation data, supported, if requested, by original dossier submissions.
- Trend analysis and revalidation data.

These records should be kept up to date and be available for inspection.

8. CHANGE CONTROL AND REGULATORY APPROVAL REQUIREMENTS

8.1 Changes to approved NIRS methods

All changes should be appropriately documented and recorded; according to approved change control protocols (see section 4.5).

In general, changes within the scope of the NIRS method should be subject to GMP inspection only. Relevant examples include the maintenance of the spectral library, replacement of equipment and other consumables with the same, including lamps, sampling devices, location and software upgrades.

To enable the dossier to be updated, extensions beyond the approved scope of the NIRS method should be subject to variation. Submissions for these changes should comply with this guidance note, including an appropriate comparability report.

For extensions of the scope of a qualitative NIRS method e.g. to include a new substance, then a statement of compliance with this guidance note and a summary comparability report would be considered sufficient.

Significant extensions would require assessment and would apply mainly to quantitative analysis.

In light of the proposed published changes to the Variations Regulation, specific advice should be obtained from Competent Authorities, pending publication of further guidance.

8.2 Method Transfer between NIRS Instruments

The aim of method transfer is to ensure that the calibration model generated on one NIRS instrument will work on another instrument. Samples analysed on the original ‘master’ instrument should give equivalent results on all additional instruments to which the calibration model is transferred. The NIRS calibration model should be demonstrated to be fit for purpose on both instruments.

The ideal approach to method transfer between NIRS instruments is to set up a global calibration model at initial registration, by including all potential instruments in the defined scope of the method. In such a case, all samples would be analysed using all instruments and all spectral data included in the calibration model. Once a calibration model is established, method transfer is likely to become progressively more difficult to achieve as more instruments are added.

One of the main problems with method transfer is a lack of uniformity of sample presentation. This is more of a problem with solid samples, for which sample orientation is critical. Different instruments will have different sample holders as well as different arrangements of detectors, which will contribute to the difficulties in achieving method transfer.

There are several commercial software packages that may be used for method transfer of NIRS models. These correct for differences in the ceramic reference standards of different instruments and perform statistical analysis of differences between results obtained. If method transfer is performed, details of the software used and the basis of the analysis performed should be given.

Method transfer of one NIRS calibration model to another would be the subject of a variation requiring assessment. The size of the transfer set of samples used to demonstrate the equivalence of the data generated on both instruments should be given and justified. The sample set should cover the full range of variation in the scope of the method.

At present, calibration transfer between NIR instruments is not considered well established and it is recommended that calibration and validation are repeated on the new instrument.

8.3 New NIRS applications for approved marketing authorisations

All new NIRS applications should comply with this guideline, however, the data to be submitted to competent authorities may be less extensive than described for non-critical applications, if the technique is well established at the testing site.

For example, for certain applications e.g. the use of NIRS as qualitative method for colouring materials and plastic primary packaging materials, it may be sufficient to submit a declaration that:

a) The NIRS method has been validated in conformity with this guideline.

b) NIRS is well established at the site (cross-referring to other marketing authorisations, as appropriate) and is subject to regular GMP inspection.

DEFINITIONS

Ambiguous conclusion	The sample is considered identical to more than one entity present in the reference library
Bias (mean of the errors)	A statistic measuring the mean of the errors between the NIRS and reference method quantitative analyte values
	$Bias = \frac{\sum_{i=1}^{n}(y_i - Y_i)}{n}$ Y = NIRS predicted value y = reference method value n = number of samples
Bootstrapping	In principle bootstrapping methods estimate the prediction error by using n observations with replacement from a data set with n samples. The average prediction ability of the samples is computed by fitting a regression model. This is repeated very often (e.g. more than 1000 times) and then the average prediction error is computed
Calibration	The process of creating a model relating two types of measured data; for NIRS methods a model that relates concentrations or properties to absorbance spectra for a set of reference samples (the reference library or the calibration set)
Calibration set	The set of samples used for creating the calibration model
Calibration Test Set	The set of samples, which are drawn from the same population as the calibration set, but were not used to generate the calibration model. In practice, the calibration set often consists of two thirds of the available sample population. The calibration test set is the remaining third
Calibration test set validation	The application of possible chemometric calibration models to the calibration test set. The derived characteristic statistical parameter is the 'Standard Error of Prediction (SEP)'
Change control protocol	A protocol listing potential future changes in the method and the actions considered necessary to prove the maintained reliability of the method
Change control test	Test used to demonstrate unchanged method reliability following a change in a method
Chemometrics	Mathematical multivariate methods to analyse or compare data
Cross-Validation	Statistical practice of partitioning a sample set into subsets such that the regression is initially performed on a single subset (calibration or training set), while the other subset (validation or testing set) are retained for subsequent use in confirming and validating the initial regression equation
Conformity	Characteristics in accordance with a certain degree of similarity (chemical and/or physical entities) to some specified standard
Factor	See Latent variable
Factorial experimental design	Two or more treatments are evaluated simultaneously in the same set of subjects through the use of varying combinations of the treatments. The simplest example is the 2×2 factorial design in which the parameters are randomly allocated to one of the four possible combinations of two treatments. Such an experiment allows studying the effect of each factor on the response variable, as well as

	the effects of interactions between factors on the response variable
Identification	Determination of the chemical identity
Internal validation	The application of resampling statistics such as cross-validation, bootstrapping and leverage correction. In summary, subsets of the calibration set data are subjected to a variety of statistical processes to identify which calibration model may best fit the available data. Each model is characterised by a statistical parameter. For cross-validation, the entire data set of samples is split into individual samples or groups of samples, which are removed individually from the rest of the samples and tested as unknowns against a calibration model constructed using the rest of the samples. The characteristic statistic is the ’Standard Error of Cross Validation (SECV)’
Latent variable or factor	Chemometric software by means of empirical correlation, when constrained by a set of calibration reference data reduce the variances in the NIRS signals to a set of functions, arbitrary generated, which are called latent variables (e.g. principle components) or factors. Inspection of the wavelength loadings of these factors should show how they contribute to the variance arising from the net analyte signal
Leverage	In chemometrics the leverage is a concept related to the Mahalanobis distance and is used to measure the influence of a sample in a model based on its similarity to the rest of the population. The Mahalanobis distance takes into account the correlations of the data set and is scale-invariant, i.e. not dependent on the scale of measurements. The leverage of a sample is the distance to the centre of all samples relative to the variability in its particular direction
No match conclusion	The sample is not considered identical to any entity in the reference library
Pass conclusion	The sample is considered identical to an entity in the reference library
PCA	Principal Component Analysis
PCR	Principal Component Regression
Performance verifications	Tests to control the instrument performance
PLS (PLSR)	Partial Least Squares (Regression)
Pre-treatment	Processing of the spectral data, with mathematical or other techniques, prior to chemometric analysis
Process Analytical Technology (PAT)	A system for analysing and controlling manufacture through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality. PAT is the sum of tools that allows enhanced control of manufacturing process, can improve process understanding and so facilitates building quality into products
Qualification	1. Characterisation based upon chemical- and physical attributes. 2. Determination of the chemical identity and the variability of the sample to the chosen model and the variability included
Qualitative method	Method with a yes or no result, e.g. identity
Quantitative method	Methods with a numerical result, e.g. assay

Ratio of performance deviation (RPD)	A statistic measuring the ratio of the standard deviation of the reference values of the calibration set (SD_{ref}) and the Standard Error of Prediction (SEP) $RPD = \frac{SD_{ref}}{SEP}$
Reference library (spectral reference library)	Database containing spectra of several batches of several substances to be tested. Spectra of unknown samples are compared with this database
Reference method	The conventional analytical method that is used to determine the concentration or property value of the samples
Resampling Statistics	Statistical methods to aid the optimisation of the calibration model by using subsets of the calibration set, e.g. cross-validation, bootstrapping, leverage correction
SEC	See Standard Error of Calibration
SECV	See Standard Error of Cross-Validation
SEL	See Standard Error of Laboratory
Selectivity	In the context of this Note for Guidance, a characterization of how selective the method is in recognizing that the sample is within the defined scope of the NIRS method. High selectivity is representative of specificity
SEP	See Standard Error of Prediction
Standard Addition	The uniform addition of the analyte the sample matrix, to enhance the net analyte NIR signal, in a controlled and understood manner
Standard Deviation (SD_{ref})	$SD_{ref} = \sqrt{\frac{\sum_{i=1}^{n}(y_{mean} - Y_i)^2}{n-1}}$ y = reference method value $y$mean = arithmetic mean of the reference method values
Standard Error of Calibration (SEC)	A statistic measuring the difference between the NIRS and reference method quantitative analyte values of the calibration set $SEC = \sqrt{\frac{\sum_{i=1}^{n}(y_{C,i} - Y_{C,i})^2}{n-p}}$ Y_C = NIRS predicted value of calibration set y_C = reference method value of calibration set n = number of samples p = number of coefficients, e.g. wavelength (MLR), principal components (PCR), factors (PLS)
Standard Error of Cross-Validation (SECV)	A statistic measuring the difference between the NIRS and reference method quantitative analyte values of the calibration set using a cross-validation method (e. g. Leave-One-Out-Method)

	$SECV = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{CV,i} - Y_{CV,i}\right)^2}{n}}$ Y_{CV} = NIRS predicted value y_{CV} = reference method value n = number of samples
Standard Error of Laboratory (SEL)	The SEL concerns to the intermediate precision (intra-lab) or reproducibility (inter-lab), whichever is applicable $SEL = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{1,i} - y_{2,i}\right)^2}{n}}$ $y_{1/2}$ = reference method value measured at different laboratory conditions n = number of samples
Standard Error of Prediction (SEP)	A statistic measuring the difference between the NIRS and reference method quantitative analyte values of the calibration test set and the independent validation set. The SEP derived from the independent validation set is considered a pivotal statistical parameter. An indication of satisfactory method performance for quality control is given by: $\frac{Range}{SEP} \geq 10$ $SEP = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{V,i} - Y_{V,i}\right)^2}{n}}$ Y_v = NIRS predicted value y_V = reference method value n = number of samples
Threshold	Limiting value, for qualitative methods, decisive for a “pass” or a “no match” conclusion
Training set	The set of samples, included in the reference library, that concern the same entity (substance or property value)
Validation set	Set of samples used in validating the model
Wavelength Correlation	The correlation between spectra, i.e. the sum of the individual correlation of absorbances of each included wavelength
Wavelength loadings	Plots of wavelengths against intensity for each derived factor of the calibration model. Such plots show the wavelengths at which the spectral variance, modelled by the specific factor or component, occurs. The wavelength loading plots indicate the parts of the spectrum that are responsible for the differences between samples explained by a particular factor or component. These can be compared with known net signal of the analyte of interest to determine if the factor significantly models the analyte signal and should be included in the chemometric model

REFERENCES (scientific and / or legal)

1 ‘Method E 1655-97: Standard practices for infrared, multivariate, quantitative analysis’, American Society for Testing and Materials, 1997. The Society, West Conshohocken, PA.

2 AACC Method 39-00: ‘Near Infrared Methods-Guidelines for Model Development and Maintenance’.

3 ‘Selectivity in Analytical Chemistry (IUPAC Recommendations 2001)’, *Pure and Applied Chemistry*, (2001), 73, pp. 1381 – 1386.