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Guideline on the use of Near Infrared Spectroscopy (NIRS) by the pharmaceutical industry and the data requirements for new submissions and variations

Draft

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Comments should be provided using this template to QWP@ema.europa.eu

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EXECUTIVE SUMMARY

Near Infrared Spectroscopy (NIRS) is a technique, usually requiring tandem chemometric statistics, with a wide and varied use in pharmaceutical, chemical, physical and process analysis. This includes identification, qualification and assay of pharmaceutical starting materials, intermediates and finished products and verification of physicochemical properties.

NIRS also constitutes one of the major techniques in Process Analytical Technology (PAT) and may also be used as part of a Real Time Release Testing (RTRT) strategy. When used as such, NIRS is underpinned by the principles of Quality by Design (QbD).

The suitability of an NIRS procedure is dependent upon many factors, including the instrumentation and applied chemometrics, as well as the sound understanding of the physicochemical basis of the measurement. This document provides guidance on the development, calibration, validation and maintenance of NIRS procedures, when used with chemometric statistics and when used for direct process monitoring.

The development and implementation of an NIRS procedure, with its interdependent stages, is iterative and ongoing, and is amenable to the application of lifecycle concepts, which allow good change control practice. Guidance on change control (whether or not within the remit of GMP), taking into account the defined scope of the NIRS procedure, is provided.

The terminology used to describe NIRS reflects its wide and varied use, both within and outside the pharmaceutical arena. A comprehensive glossary is therefore provided to support this guideline.

1. Introduction

To aid the narrative of this guidance, the following key terms are used:

NIRS method: describes the key elements, principally within the NIRS apparatus, which enable NIRS measurement of the analyte of interest

NIRS model: describes how the NIRS spectral data measured using the NIRS method are related to the analyte of interest, generally employing chemometric software.

NIRS procedure: describes how the NIRS method and model are used for the intended purpose, within its defined scope (see section 4.1.1)

NIRS allows for fast, direct measurement of materials with little or no sample preparation, usually generating complex spectra, which can only be interpreted easily by the use of chemometric models. These are developed using carefully selected and representative samples, which have in turn been qualified by a reference analytical method, using analytical reference standards. Consequently, NIRS is not generally used as a 'primary' analytical method.

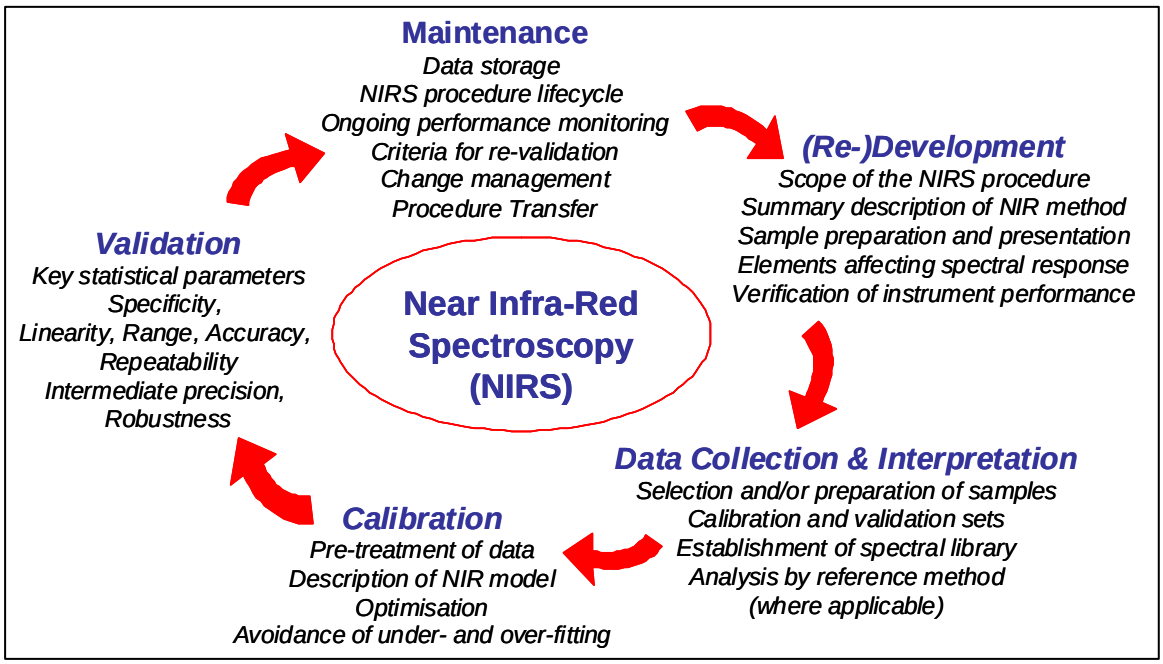
In general, NIRS procedures used for the release testing of drug substances or drug products need to be developed and validated in conjunction with the reference methods. As NIRS procedures cannot be repeated easily by official control laboratories, the reference methods and corresponding specifications should remain in the authorised specifications, with an indication that these methods would not be used for routine batch release.

For PAT NIRS procedures, e.g. dynamic process monitoring of a powder blend, it may not be possible to refer to a conventional reference method, if this is unavailable for justified reasons e.g. the potential for sampling errors.

It is recognised that the development and implementation of an NIRS procedure is iterative and that the stages are interdependent. The main stages in developing and establishing NIRS procedures are summarised in Figure 1. Some of these stages may not be necessary for all procedures such as variations or PAT applications, for which the absence of information for these stages may then be justified.

It is possible to update calibration models of NIRS procedures as new data become available following the purchase or production of new analyte batches. This is considered good practice and is recommended. For guidance on the implementation of changes, see Section 7.

Figure 1. The iterative nature of NIRS



2. Scope

This guideline describes the regulatory requirements for marketing authorisation applications and variation applications submitted for medicinal products for human or veterinary use, which include the use of NIRS.

NIRS differs from conventional analytical techniques such as HPLC or GC because chemometric techniques are generally (although not exclusively) required for interpretation of the analyte signal.

NIRS is described in the European Pharmacopoeia; however a single reference to the Ph.Eur. general chapter on NIR spectroscopy (Ph.Eur. 2.2.40) as a sole description for the NIRS procedure is insufficient to support the use of such a procedure in marketing authorisation applications or variation submissions.

This guideline outlines the requirements for applications in which NIRS is used for qualitative and quantitative analysis or where it is used as a process analytical technology (PAT) for monitoring and controlling drug substance synthesis and finished product manufacturing processes. Approaches other than those described in this guidance may be used, if appropriately explained and justified.

The chemometric principles described within this guideline may also be applicable to other analytical techniques.

3. Legal basis

This guideline should be read in conjunction with Directive 2001/82/EC, as amended and Directive 2001/83/EC, as amended.

This guideline should be read in conjunction with:

- Ph. Eur. Monograph 2.2.40.
- ICH Q2(R1) Guideline on Validation of Analytical Procedures (CPMP/ICH/381/95)
- VICH Guidelines GL1 & GL2 on Validation of Analytical Procedures (CVMP/VICH/590/98 & CVMP/VICH/591/98)
- CHMP and CVMP Notes for Guidance on Process Validation (CPMP/QWP/848/96 & EMEA/CVMP/598/99)
- ICH Q8: Guideline on Pharmaceutical Development
- ICH Q9: Guideline on Quality Risk Management
- ICH Q10: Guideline on Pharmaceutical Quality System
- ICH Guideline Q8, Q9 and Q10 - questions and answers (CHMP/ICH/265145/2009)

4. General requirements

4.1. Development

4.1.1. Establishing the scope of the NIRS procedure

The scope of the NIRS procedure (i.e. how the NIRS method and NIRS model are to be used for the intended purpose) should be clearly identified in any application in which NIRS is used. The scope of the NIRS procedure should include details of the NIRS method (i.e. the key elements that enable NIRS measurement), the NIRS model (e.g. how the NIR spectral data are related to the analyte or property of interest) as well as any limitations of the method (e.g. operating range of validity with respect to analyte concentration, probe position, wavelength range, chemometric algorithm used etc). The definition of the scope of the NIRS procedure is particularly important for the consideration of how future changes to the procedure may be implemented from a regulatory perspective (see Section 7).

NIRS has a wide range of qualitative and quantitative applications and its use requires a sound understanding of the physicochemical basis on which its measurements rely and of the instrumental and chemometric principles involved. The applicant should identify any assumptions made during procedure development.

The NIRS signal may be directly attributed to the analyte of interest or may be an indirect measurement correlated with light scattering effects. The applicant should discuss the scope and purpose of the NIRS procedure and show it to be relevant to the analyte or property under consideration.

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

The complex informative content of the NIRS signal often requires chemometric techniques to visualise and extract relevant information from the spectra, or to show that the spectra are correlated to a signal measured with the reference method. Chemometric data analysis and modelling are usually performed using statistical software packages. In general, these work by correlating the variance in the NIRS signal 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; therefore chemometric models should always be validated with an independent set of samples.

4.1.2. Summary description of the NIRS method

Summary details of the NIRS method should be provided, including the instrument measurement mode (e.g. reflectance, transmission, transflectance), the principle of the monochromator (e.g. grating, FT-IR), the wavelength (wavenumber) range used, the detector type (e.g. silicon, lead sulphide), the optical bandwidth, the spectral resolution, wavelength accuracy and precision, the signal to noise ratio, a description of any sampling devices and details of any other additional components or controls considered necessary for the proposed procedure. The means of data collection, analysis and associated software packages should also be described.

4.1.3. Feasibility study

The feasibility of using NIRS should be considered in the development of new procedures to demonstrate that it is suitable for the intended purpose. Such a feasibility study may include (but is not limited to), the determination of a suitable NIR response, investigations into specificity and matrix interference and the examination of the effects of sample handling and preparation.

4.1.4. Variables affecting spectral response

Background physical and chemical variables, which may affect the spectral response, may be present.

It is not possible to list all possible variables, but these may 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.

Each known potential variable that may affect the spectral response should be considered and discussed in turn and either shown to be insignificant or controlled satisfactorily (supported by appropriate data).

4.2. Data collection

4.2.1. Sample preparation and presentation

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

The interface between the sample and the NIRS detector should be described. The impact of possible variations of the presentation on the NIRS response should be discussed, supported by appropriate data, and, if shown to be significant, demonstrated to be controlled satisfactorily.

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

The spectral range employed and the number of scans recorded per sample should be stated and justified.

4.2.2. Sample population

Samples should be independent. The applicant should define what they understand to be a 'sample' and their definition of an 'independent sample'. These definitions should be justified with respect to the parameter that the intended model is proposed to predict.

Samples for NIRS analysis should be representative of the production process and should be collected according to acceptable procedures for sampling. Samples that are representative of the commercial process, which were obtained during development and pilot scale production may also be utilised. Justification should be given to support the choice of samples.

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

- concentration of the analyte of interest
- particle size
- material suppliers
- water content
- residual solvent content
- qualitative and / or quantitative variations in the matrix (e.g. excipient grade, formulation)
- process variation (e.g. samples collected over an extended period)
- sample age
- temperature

4.2.3. Pre-treatment of data

Given that NIR spectra are affected by physical parameters such as particle size and sample presentation, raw NIR spectra are often treated mathematically prior to development and testing of the calibration model. Such treatments include (but are not limited to) normalisation and derivation, which are performed in order to remove unwanted sources of variation from the data prior to treatment and to enhance spectral features.

Caution must be exercised when performing any pre-treatments because artefacts can be introduced or essential information lost. Any pre-treatment of data should be documented and justified.

4.2.4. Analysis by the reference method (when applicable)

For NIRS procedures for which a reference analytical method is used, the samples used for NIRS calibration and validation require authentication or quantitative values to be assigned by the reference

method. Ideally, reference measurements should take place at around the same time as NIR scanning.

When a reference method is used, 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.5.21 data requirements.
- details of the validation of the analytical procedure according to the Module 3.2.P.5.31 data requirements and the ICH Q2(R1) Guideline on Validation of Analytical Procedures (CPMP/ICH/381/95). For Veterinary applications reference is made to the VICH Guidelines GL1 & GL2 Validation of Analytical Procedures (CVMP/VICH/590/98 & CVMP/VICH/591/98).
- details of relevant reference standards and materials according to the Module 3.2.P.61 data requirements.

4.2.5. Establishment of a spectral reference library

The composition of the spectral reference library should cover the scope of the NIRS procedure and should be subject to a change management system (subject to GMP inspection).

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

For qualitative analysis, where the spectral reference library may be 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 number of sub-libraries should be described and justified. Measures should be taken to avoid using the wrong library. The use of only one library can avoid the possible error of using the wrong library.

4.3. Data interpretation

4.3.1. Description of the NIRS model

The NIRS chemometric model (if used) should be described fully. Further information in relation to the model is given in the sub-chapters for 'Qualitative Procedures' and 'Quantitative Procedures' (Sections 5.3 and 6.3).

4.3.2. Statistical spectral quality test

Before an NIRS model may be applied to a sample, a statistical spectral quality test (e.g. a model suitability diagnostic) should be performed, to determine whether the characteristics of the sample fall within the range of variation for which the model was calibrated and validated. In practice, such tests (e.g. Hotellings T2 or Distance to Model (DModX) plots) show whether the spectra for the sample fall within a pre-defined range of variation or if the sample is an outlier. The steps taken to address spectral outliers should be described (see Section 4.3.3).

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

If a sample fails this initial spectral quality test, it is poor scientific practice to test the sample using the developed calibration model regardless, since a 'false' positive or otherwise invalid result would be obtained.

A clear description of the spectral quality test should be described in any procedure involving the use of NIRS. Such a test will often be performed automatically by the computer software but should nevertheless be described and shown to be understood by the applicant.

4.3.3. Outliers in sample data

Any suspected outliers in the sample data (NIRS or reference data, where applicable), which are to be included in the calibration, calibration test or validation data sets should be investigated and any exclusions justified.

The term 'outlier' within this guideline refers to unexpected results or results outside of the specified range. An outlier may be a 'spectral outlier' (spectral data outside but prediction result within the range), a 'reference outlier' (spectral data within the range but reference value outside) or both spectral and reference data beyond the proposed scope of the NIRS procedure.

In practice, there may be several reasons for outliers, e.g. a sample belonging to a different population to the rest of the samples, instrument malfunction, reference method failure or transcription error. In this sense, such a result may not necessarily be a false observation but merely an observation that is different from the rest and that could have an undefined influence on the results of the analysis.

If a sample is shown to be an outlier because of characteristic properties, the sample should be verified using an appropriate alternative analysis. After confirmation of authenticity, the sample may be included in the spectral reference library and the model should be re-validated so as to include this source of variation. This is an important part of the NIRS procedure lifecycle (see Figure 1, Section 1 and Section 7) and it is important to ensure that the procedure is updated and optimised.

At any stage of NIRS procedure (re-)development, calibration or validation, systems and procedures should be in place to ensure that the handling of outliers in the data is performed properly. Such procedures should be described.

4.3.4. Out of Specification (OOS) results in routine batch analysis

An OOS result for routine batch analysis by NIRS analysis should result in the investigation of the affected batch under the company's pharmaceutical quality system. A rejection should be performed if the OOS result is confirmed by a failure investigation.

A batch should not be released based on an OOS NIRS result and a within-specification result when tested using the reference method (if available).

If, on investigation, the affected batch complies with the specification using the reference analytical method, then this may indicate that the NIRS procedure has not been fully developed. The NIRS procedure may then be updated as necessary (as per the NIRS procedure lifecycle concept, see Figure 1) and re-analysis undertaken such that the batch may be released within specification for both the NIRS procedure and the reference method of analysis.

4.4. Calibration

Specific requirements for calibration are described in the sub-chapters for 'Qualitative Procedures' and 'Quantitative Procedures'.

4.5. Validation

Validation of NIRS procedures should comply with the data requirements for Module 3.2.P.5.32 and the guidance given in ICH Q2(R1) Guideline on Validation of Analytical Procedures (CPMP/ICH/381/95). For veterinary applications reference is made to the VICH Guidelines GL1 & GL2 Validation of Analytical Procedures (CVMP/VICH/590/98 & CVMP/VICH/591/98).

The validation set of samples (for external validation) should be completely independent of the calibration set (see 5.5 and 6.4).

A comparison of results obtained by analysis of the same set of samples by the NIRS procedure and the reference method (if applicable) forms part of the validation of NIRS, along with independently determined parameters, such as intermediate precision. In all cases, whether a reference method is used or not, the acceptance criteria for validation should be specified and justified with reference to the intended purpose of the NIRS procedure.

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

If the NIRS procedure is being registered as a variation to a marketing authorisation for which a reference method is already approved, then a summary of the validation data for the reference method, in compliance with the current (V)ICH guidance on validation of analytical procedures, should be provided.

Specific requirements for validation are described in the sub-chapters for 'Qualitative Procedures' and 'Quantitative Procedures'.

4.6. NIRS in Process Analytical Technology (PAT) applications

In the context of PAT applications, almost all NIRS procedures are specific to the nature of the individual manufacturing processes. It is therefore not appropriate to prescribe exact requirements for such procedures in this guideline.

The general data requirements described in this guideline are also applicable to NIRS PAT procedures and should particularly take into account the intended purpose and scope of the procedure.

It is important that the NIRS methods are described in detail, with specific information in relation to the applied PAT application (e.g. spatial placement of the devices, structural peculiarities of the manufacturing facility and the nature and extent of sampling).

The amount of information required for an NIRS PAT procedure will depend on its intended purpose. Any reduction in the extent of the description or control of the procedure (e.g. sampling, data pre-treatment, NIRS model, calibration, validation, etc) should be fully described and justified scientifically.

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

Examples of NIRS in PAT applications include:

- drug substance manufacturing process steps such as chemical reaction kinetics, crystallisation, drying and milling
- drug product manufacturing process steps such as granulation, blending, tablet hardness and coating

In the case of the monitoring of a powder blend for homogeneity, the blend may be monitored in terms of the measurement of the change of the NIR signal (e.g. its standard deviation) over time, rather than in relation to a reference method such as HPLC.

4.7. Summary of general data requirements

The following data requirements apply to both qualitative and quantitative NIRS procedures. Additional specific requirements for these are outlined in Sections 5 and 6 respectively.

- the scope of the NIRS procedure (see definition and explanation in Section 4.1.1)
- details of the composition of the calibration set, calibration test set and validation set of samples, with justification
- description of the reference analytical method (where applicable)
- report(s) of the calibration and validation of the NIRS procedure and reference method (as applicable, with summary details if already approved)
- details of how the NIRS procedure lifecycle will be managed (e.g. in accordance with this guideline)

If an applicant wishes to use terminology other that that described in this guideline, the terminology should be fully and clearly explained (e.g. using a glossary).

5. Qualitative procedures

5.1. Development

NIRS has a wide range of qualitative applications, almost all of which could be divided into the three areas of identification, qualification and conformity checks.

Identification and qualification

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 quality attributes of one chemical entity (e.g. particle size, polymorphs).

To allow differentiation, this guideline uses the term identification as referring to chemical structure only and qualification as referring to chemical and physical attributes.

If identification and/or qualification are based on more than one analytical method, then it should be clear, if applicable, which reference method(s) will be replaced by the proposed NIRS procedure.

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. It may be necessary to apply chemometrics in order to compare the data and to draw conclusions (pass, no match or ambiguous). The appropriate confidence level of the conclusion should be justified.

If an ambiguous conclusion is obtained, the NIRS procedure 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 procedure. Interfering substances or grades of substances may be identified as one single entity if desired (e.g. different grades of lactose).

The classification of a substance can be performed in several stages. For example, the identification of a chemical identity or a group of related substances may be performed, followed by the use of more selective libraries 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, the library for qualification measures how well a sample fits in with a library derived from samples chosen to represent the defined variability of a chemically identical substance.

Where the NIRS procedure on its own is not sufficient to identify or qualify a substance, it should be supplemented by other different analytical procedure(s) (e.g. chemical reaction or chromatographic methods), so that the tests taken together ensure, as far as possible, specificity.

Conformity checks (PAT, dynamic process monitoring or trend analysis)

This guideline 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. Such conformity checks refer to process characterisation or trend analysis, for example the determination of the endpoint of a process by monitoring the change in NIRS signal. These NIRS procedures are based on processes that yield continuous values.

Conformity NIRS procedures may also be known as ‘dynamic process monitoring’, trend analysis or Process Analytical Technology procedures (PAT, see Section 4.6) and will often not involve the use of a reference analytical method because of difficulties in sampling for reference analysis.

Conformity checks should generally be treated in a similar way to qualitative procedures with respect to calibration and validation, however the extent of the calibration and validation work performed will depend on the intended purpose of the procedure. For example, validation of a ‘moving block standard deviation’ procedure will focus mainly on a specific end-point, supported by sound rationale and analytical evidence of the procedure’s predictive ability (see Section 4.1).

5.2. Data collection (qualitative procedures)

5.2.1. 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 procedure. All samples should be verified with the approved reference methods where applicable, or authenticated by appropriate means (certificate of analysis or relevant testing). The validation of the NIRS procedure should demonstrate that spectra of an acceptable minimum number of batches have been included in the spectral library and that these batches are sufficiently representative to cover the normal variation of the substance.

Where laboratory or pilot scale samples are required to present wider variability than that shown for production samples, such samples should be prepared using the same manufacturing procedure as used for routine batches, unless otherwise justified. 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 NIRS procedure for routine use.

For procedures used to identify or qualify substances on receipt, samples from all known potential suppliers should be incorporated into the library.

For conformity checks (in-process controls and monitoring purposes), the sample population should be justified with respect to the intended purpose of the procedure.

5.2.2. 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 interference by the matrix of the analyte signal of interest. In general, the more complex the sample matrix, the more samples will be required to cover the statistical population.

The number of samples per batch and the number of batches used for calibration and validation should be sufficient to cover normal production variation and should be fully justified.

5.2.3. Composition of sample sets

In order to develop, optimise and validate a calibration model for a typical qualitative NIRS procedure used for identification or qualification, two sets of samples are required:

- a calibration set for creating the calibration model
- an independent validation set for (external) validation of the proposed chosen model

The calibration set of samples contains all those samples proposed for inclusion into the spectral library. In the simplest form of spectral library, samples of all material groups (i.e. all materials used at a particular site) are included in one library and chemometric analysis is applied to this library. Since this may not always provide adequate specificity, sub-libraries are often used, containing all samples of a particular class, to ensure the required specificity. Identification and qualification may therefore be an iterative process, with identification of a substance in the first instance using the main spectral library followed by qualification of, for example, the polymorphic form of that substance using a sub-library.

Each set of samples should be representative of the intended scope of the NIRS procedure and include samples covering the full range of potential variation in the sample population.

The independent validation set of samples should be entirely independent of those samples used to build the spectral library and should include qualitatively positive and negative samples.

The selection of an appropriate calibration model may be aided by so-called ‘internal validation’ methods. ‘Internal validation’ is the application of resampling statistics to cross-validate and provide an ‘internal check’ of the performance of the model for the purposes of optimisation. A subset or subsets of the spectral reference library data are subjected to a variety of statistical processes to identify which calibration model (generated by the software) may best fit the available data.

For conformity procedures, the principles given above apply; however, samples would be expected to cover the range of variability shown within the process being monitored and to give correct determination of an end point. Positive and negative results would be expected to be included in the independent validation set of samples, to ensure that the NIRS procedure is fit for purpose (e.g. homogeneity and in-homogeneity in a blending process, with the inclusion of samples that have been under-blended or over-blended to the point of de-mixing).

5.2.4. Outliers

Identified outliers in a spectral reference library should be investigated and should be excluded only based on valid analytical reasons. These should be documented and justified. See also Section 4.3.3.

5.3. Calibration

Examples of calibration algorithms 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), Supported Vector Machines (SVM) and correlation algorithms such as distance-match.

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

It is almost always necessary to determine thresholds, confidence limits or tolerances for the proper identification and/or qualification of samples. The relevant values for the chosen calibration model should be stated, explained and justified in the validation report.

5.4. Optimisation

In general, the optimisation of a qualitative procedure is confined to the selection of the samples included in the model and the choice of pre-treatments and the calibration algorithm.

5.5. Validation (internal and external)

5.5.1. General considerations

The objective of 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 where necessary, a discrete set of samples. This internal validation step should demonstrate that all samples of the spectral reference library are identified or qualified according to the scope of the procedure, within the defined thresholds, confidence limits and/or tolerances.

External validation of the procedure should demonstrate the performance of the chosen model using an independent validation set consisting of samples that were not used in the creation of the spectral reference library.

The applicant should demonstrate that the NIRS model is suitable for the intended purpose by the means of appropriately defined and justified confidence limits. Alternative thresholds and/or statistical

parameters may be used to evaluate the performance of the model, which should be stated, fully explained and their suitability for the intended purpose should be justified.

Qualitative procedures should be validated for a minimum of specificity and robustness.

5.5.2. Specificity

The extent of specificity testing depends on the intended NIRS procedure. A lack of specificity may be compensated for by other supporting analytical procedures.

Independent samples of substances represented in the spectral reference library, but not used to create it (e.g. different batches, blends), must be tested and all should be 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 (if available) should be included in the validation set, unless their absence is justified. A risk assessment of the goods-in and manufacturing operations should be used to justify the analogues and challenges presented to the model.

Where applicable (e.g. for qualification procedures), validation should include challenge with different grades of the same substance, anhydrous/hydrated material, different polymorphs or material supplied by different vendors.

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

The composition of the independent (external) validation set of samples should be described unambiguously and should be justified.

5.5.3. Robustness

Effects of relevant variables 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, different 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.

The use of Design of Experiments (DOE) may be considered to maximise the information available.

6. Quantitative procedures

6.1. Development

It is in the interests of the developer to perform a feasibility study to determine the likelihood of success of any quantitative NIRS procedure and to outline the minimum requirements that should be built into the development and validation protocols (e.g. the consideration of tablet weight as a variable in the determination of uniformity of dosage units to ensure the measurement is total content rather than concentration).

6.2. Data Collection (quantitative procedures)

6.2.1. Sample collection and population

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 (when feasible). At the same time, the possibility of the introduction of undesirable correlations and systematic errors should be considered, taking account of the known NIRS signal of the analyte of interest.

The distribution of samples should be evaluated with respect to the intended purpose of the procedure. For cases in which the NIRS procedure is used to test whether a sample meets a specified limit, it may be acceptable to include more samples around the proposed specification limit. This should be explained and justified.

The choice and number of samples should be justified and if possible, supplemented using DOE correlated with the intended purpose of the procedure.

6.2.2. Number of samples

Calibration algorithms are generally based on the correlation of variance in the NIRS signal to a number of principal components, constrained by a set of calibration reference data. To avoid bias, the number of samples used to develop the calibration model should be very much greater than the number of principal components used (or equivalent, where applicable). In all cases, the number of samples used to develop the calibration model should be justified.

The number of samples to be included in order to create a valid calibration model for quantitative analysis will depend on the complexity of the sample matrix and/or interference by the matrix of the analyte signal of interest. In general, the more complex the sample matrix, the more samples will be required to cover the statistical population. For example, if the sample matrix consists of two simple ingredients only, the number of samples required will be lower than if a multi-ingredient, complex system is to be analysed. For the latter, a complex chemometric model, with more principal components, may be required, for which a greater number of samples will be necessary to ensure its validity.

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

6.2.3. Composition of calibration set, calibration test set and validation set of samples

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

- the calibration set for creating the calibration model
- the calibration test set for optimisation and choice of calibration model (if used)
- the independent validation set for external 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 NIRS procedure.

The optimisation/choice of the calibration model is normally undertaken by so-called 'internal validation' methods. These methods involve the application of re-sampling statistics to cross-validate and provide an 'internal validation' of the performance of the model for the purposes of optimisation. A subset or subsets of the spectral data are subjected to a variety of statistical processes to identify which calibration model (generated by the software) may best fit the available data.

The calibration test set is used to provide the first 'test' or check of the validity of the model. The calibration test set does not represent independent validation of the NIRS procedure (which must be carried out using an entirely independent set of samples), since the samples are taken from different batches within the same (historical) population of batches. In practice, the calibration set often consists of two thirds of the available sample population and the calibration test set is the remaining third, however this is not always the case and the calibration set should always contain a sufficient number of samples to ensure that the generated calibration model is robust. The applicant should give the rationale for the composition and number of samples in the calibration and calibration test sample sets and justify their suitability.

The validation set (for external validation) is an entirely independent third set of samples, which is not taken from the same (historical) population as those batches used to generate the calibration model. In principle, this external validation set should cover the full range of variation in the sample population and should include production-scale batches, where possible. The number, scale and composition of batches included in the validation sample set should be discussed and justified.

The validation set of samples is used to validate the calibration model and is used to generate the statistical parameter, the Standard Error of Prediction (SEP), which is an indicator of the validity and predictive ability of the calibration model. An SEP will also have been generated for the calibration test set, however this is used as an initial indicator of the predictive validity of the model only. The SEP for the external validation set of samples is the pivotal statistical parameter for the model.

An analyst may choose not to apply 'internal validation' methods during development and optimisation of a calibration model. In such cases, a calibration set and an independent validation set of samples would be the minimum requirement.

6.2.4. Analysis by the reference method

The performance of a quantitative NIRS procedure is dependent on the performance of the reference method (if used). Poor precision and accuracy of the reference method will limit the performance of

the NIRS procedure. It is important that care is taken to ensure that uncertainty in the reference method is low in relation to the intended performance of the procedure.

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 method and the NIRS procedure.

6.3. Calibration

6.3.1. Software

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

Using 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. The result of this correlation may be presented in different ways depending on the algorithm used (e.g. latent variables for PLSR or principal components for PCA).

6.3.2. Selection of Principal Components

The number of principal components (or similar, which describe the variability in the data) to be used in the calibration model is of critical importance to avoid under or over fitting of the data.

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

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

- co-linearity
- minimal contribution to the data variance arising from spectral variations of the analyte of interest
- contribution to the data variance arising not from the spectral variations of the analyte of interest, 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. Loadings plots (describing the variation explained by each component) may be useful when choosing the number of components to use. For procedures in which principal components are not used (e.g. neural networks), the chosen key parameters should be stated, explained and justified.

Once selected, the proposed calibration model should be characterised, by graphical and statistical means, the characteristic statistic being the Standard Error of Calibration (SEC) or equivalent. Parameters such as bias and intercept may also be reported.

6.3.3. Optimisation of the NIRS model

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 procedure. Optimisation of calibration models may be

performed by 'internal validation' methods as described in Section 6.2.3. These methods are used to aid assessment of the suitability of the calibration model in its ability to predict the correct quantitative result.

It is accepted that the use of resampling statistics for optimisation is a rapidly developing field and that more appropriate statistical processes may be available, particularly relating to the assessment of under- and over-fitting. Any process used should be explained and justified.

For internal (cross) validation methods of optimisation, the characteristic statistic is usually the Standard Error of Cross Validation (SECV). Other statistical parameters such as bias or the coefficient of variation may also be used. Any statistical parameter(s) used during optimisation of the model should be stated, explained and justified.

6.4. Validation

6.4.1. General considerations

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

Since quantitative NIRS analysis relies upon reference data obtained from a reference method or very rarely, samples of known composition in order to impart meaning to the sample spectroscopic data collected, a statistical acceptance criterion is used as a measure of the model's ability to predict the correct quantitative result. This is the SEP, for the independent validation set of samples (external validation).

Quantitative NIRS procedures should be validated with respect to the following parameters:

6.4.2. Standard Error of Prediction (SEP)

The SEP and the SEP/range ratio should be determined for the external validation set. The value of these parameters should be explained, discussed and justified with respect to the intended purpose of the NIRS procedure. These are considered pivotal statistical parameters. Other equivalent terms should be explained and justified.

It may be helpful or appropriate to report and justify the SEP/Standard Error of Laboratory (SEL).

6.4.3. Specificity

The specificity of an NIRS procedure is dependent upon its intended purpose, scientific basis and scope.

For specificity, the procedure 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 (see also section 4.3.2 Statistical spectral quality test).

The following may be used as supportive evidence of specificity:

- reference to the feasibility study data demonstrating a suitable NIR response based on the known NIR characteristics of the analyte

- comparison of the loadings plots for the components used to develop the chemometric model, against the known NIR characteristics of the analyte (where applicable)
- validation data to demonstrate accuracy and robustness

6.4.4. Linearity

To demonstrate linearity, it is required that samples in the validation set 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 (if applicable). The correlation coefficient and analysis of residuals (indicators of linearity), should be explained, justified and supported by graphical representation.

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

6.4.5. Range

The range should be confirmed by use of a suitable validation set which matches in extent the proposed range for the intended use of the procedure. Validation samples having analyte content outside of the calibration range should appear as outliers when tested by the NIRS procedure.

6.4.6. Accuracy

Accuracy should be established across the specified range of the NIRS procedure and should be appropriate for its intended use. In some case, the NIRS procedure may have a higher error than the reference method. In such cases, limits may be set that are tighter than those set for the reference method.

6.4.7. Precision

Precision and the SEP should be appropriate for the intended use of the NIRS procedure. Bias should be as close to zero as possible.

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

Dependent upon the nature by which 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.

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 reference data for quantitative NIRS procedures 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 an NIRS procedure should cover chemical and physical variables, dependent upon the purpose of the procedure and the conditions employed for sampling. These variables may include temperature and humidity, sample handling and instrument changes as discussed in Section 4.1.4 (Variables affecting the Spectral Response). The use of DOE may be considered to maximise the information available.

Robustness should be addressed within the scope of the NIRS procedure. If this has been considered, 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, validation data for the determination and assurance of robustness should be provided.

6.4.9. Limits of detection and quantification

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

7. NIRS procedure lifecycle and post-approval requirements

7.1. Management of the NIRS procedure lifecycle

It is recognised that NIRS procedures (qualitative or quantitative) will evolve over time (see Figure 1, Section 1). The applicant should indicate how they will manage the NIRS procedure lifecycle in the initial application.

The scope of the NIRS procedure should be clearly defined in the initial application (see definition in Section 4.1.1). This is critical with respect to how post-approval changes to the NIRS procedure should be implemented.

Any extension of the scope of the NIRS procedure should be implemented by variation application only.

7.2. Changes to approved NIRS procedures

Changes (both planned and unplanned), which might affect the performance of an NIRS procedure, may necessitate re-calibration and/or re-validation of the NIRS model to demonstrate continued model suitability. All changes should be validated accordingly, appropriately documented and recorded according to valid change management protocols.

7.2.1. Changes within the defined scope of the NIRS procedure

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

Changes should be fully documented, and include appropriate re-validation and comparability reports to show that the revised NIRS procedure is consistent with the approved procedure. A risk assessment should be conducted to determine the risk associated with the change being made.

For qualitative NIRS procedures, suitable change management tests should be in place for each NIRS procedure and spectral reference library (where applicable). A change management test should be

composed of a minimum of two standard sets of samples (i.e. two classes or substances) for which separation is most critical. If the NIRS procedure does not comply with the change management test (meaning that the procedure is unable to distinguish between the two sets of samples), the model should be fully re-validated. The suitability of the change management test should be subject to periodic re-evaluation.

Quantitative NIRS procedures should only be used within the calibrated concentration range and using conditions defined in calibration. It may be appropriate to add sample observations into the calibration model (within the calibrated range detailed in the defined scope of the NIRS procedure) via model updates. Such changes require re-validation and documentation should be available for GMP inspection.

7.2.2. Changes outside of the defined scope of the NIRS procedure

To enable the dossier to be updated, extensions outside of the approved scope of the NIRS procedure are subject to variation application, including an appropriate comparison of the updated NIRS procedure with the current procedure and/or reference analytical method, if applicable.

For extensions of the scope of a qualitative NIRS procedure e.g. to include a new substance (previously not included) into a spectral library, a statement of compliance with this guideline and a comparison of the updated NIRS procedure with the current procedure would be considered sufficient.

For quantitative analysis, extensions of the scope of NIRS procedures include for example, changes of ranges and/or specification limits. Variation applications for such changes require evidence of re-calibration and validation of the NIRS model.

7.3. NIRS procedure transfer between NIRS instruments

The aim of NIRS procedure transfer is to ensure that the calibration model generated on one NIRS instrument will work on another instrument, based on the validation parameters detailed in Sections 5.5 and 6.4 of this guideline. Samples analysed on the original 'master' instrument should give equivalent results on all additional instruments to which the calibration model is transferred. The following parameters are essential to judge the similarity of instruments:

- hardware (e.g. identical spectrometer type and measuring set-up)
- software (including mathematical algorithm and how the spectra are treated in the calibration model)
- interfaces (e.g. probes and waveguides)

Depending on the scope of the NIRS procedure (e.g. qualitative, trend analysis or quantitative) and the degree of similarity of the instruments involved, it may be necessary to apply one of the following options:

1. Where there are NIRS method changes only:

   It may be possible and sufficient to include a mathematical compensation in the applied software of the NIR instrument to ensure that identical spectral responses are achieved for a representative set of reference samples when tested by all instruments. In general, a bias and or slope or vector correction is undertaken.

2. For all other cases, for which mathematical compensation is insufficient:

Calibration and validation should be repeated and confirmed on the additional instrument(s).

Calibration transfer models may be developed using a small but representative number of calibration samples that are run on both instruments (the master and the additional instrument). A convenient method for choosing samples is one that is based upon their good multivariate leverage. In this method, samples are selected that have a large influence on the calibration model. Depending on the complexity of the multivariate model, a smaller representative number of samples (in comparison with the number used to calibrate and validate the model originally) should be sufficient to support model transfer between instruments.

In the event that the master instrument is no longer available, an appropriate number of samples should be justified and used to build the calibration model on the additional instrument(s).

In both cases (1) and (2), the transfer of an NIRS procedure to another instrument should be the subject of an appropriate comparability protocol.

The comparability protocol for NIRS procedure transfer(s) may be submitted with the initial application as a 'post approval change management protocol' if changes are foreseen in the applicant's strategy. Alternatively, the comparability protocol may be submitted at the time of the variation application to register the transfer itself. The protocol should include criteria that have been justified to be acceptable to demonstrate a satisfactory transfer.

8. Glossary

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 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
Calibration	The process of creating a model relating two types of measured data. For NIRS, 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 management protocol	A protocol listing potential future changes in the NIRS procedure and the actions considered necessary to prove the maintained reliability of the procedure
Change management test	Test used to demonstrate unchanged NIRS procedure reliability following a change
Chemometrics	Mathematical multivariate methods to analyse or compare data
Cross-Validation	See Internal validation
Conformity	Characteristics in accordance with a certain degree of similarity (chemical and/or physical entities) to some specified standard
Design of Experiments (DOE) (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. Subsets of the calibration data set 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	See Principal component
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
Loading plot	The loading plot for each principal component indicates the magnitude (small or large correlation) and the manner (positive or negative correlation) of how each original measured variable (e.g. wavelength of the NIR spectra) contributes to the variance seen for the analyte signal
NIRS Procedure	Describes how the NIRS method and model are being used for the intended purpose.
NIRS Method	Describes the key elements that enable the NIRS measurement of the analyte of interest. This includes for example, the equipment and spectrophotometer type (e.g. FT, grating etc), the sample measurement interface (e.g. probe, sample stage etc), the number of scans or measurements and the spectral range of the instrument.
NIRS Model	Describes how the NIR spectral data are related to the analyte property of interest or the intended use of the procedure.
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
Principal component or latent variable	Principal components are calculated by means of chemometric software from a set of original variables (e.g. NIR spectra) by linear transformation of the original variables into a lower dimensional space. The principal components have the characteristic that a maximal amount of information about the original variables is retained

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 within the defined variability of the material
Qualitative procedure	Procedure with a yes or no result, e.g. identity
Quantitative procedure	Procedure 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
Re-sampling Statistics	Statistical methods to aid the optimisation of the calibration model by using subsets of the calibration set, e.g. cross-validation
SEC	See Standard Error of Calibration
SECV	See Standard Error of Cross-Validation
SEL	See Standard Error of Laboratory
SEP	See Standard Error of Prediction
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 procedure 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 procedure and reference method quantitative analyte values of the calibration set using a cross-validation 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 procedure 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.

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{V,i} - Y_{V,i}\right)^2}{n}}$$

Y_{v} = NIRS predicted value
y_{V} = reference method value
n = number of samples

Threshold

Limiting value, for qualitative procedures, decisive for a 'pass' or a 'no match' conclusion

Validation set

Independent 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

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