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▲{856} NEAR-INFRARED SPECTROSCOPY

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1. INTRODUCTION

Near-infrared (NIR) spectroscopy covers the electromagnetic field wavelength range from 780 to 2500 nm, with the frequencies expressed in wavenumbers (about 12,821–4000 cm⁻¹). NIR spectroscopy is a vibrational spectroscopic technique that measures the absorption of radiation in this wavelength range that is resonant with frequencies from molecular dipole changes in the sample; thus, NIR spectroscopy is related to infrared (IR) spectroscopy. The NIR spectroscopic region is dominated by overtone and combination frequencies of the molecular fundamental harmonic frequencies in the mid-IR region. The molar absorptivities in the NIR region tend to be much lower than those in the IR region, and the radiation can penetrate several millimeters into the material, giving rise to a wavelength-dependent scattering signal overlapping the molecular absorbance signals.

Not all materials absorb NIR radiation, and materials such as glass are relatively transparent. These attributes of NIR spectroscopy have been shown to be useful in the area of pharmaceutical analysis for both qualitative and quantitative applications. For further discussion of the theory and applications, see *Near-Infrared Spectroscopy—Theory and Practice* {1856}, which may be a helpful, but not mandatory, resource.

The instrument qualification tests and acceptance criteria provided in this chapter may not be appropriate for some instrument configurations. In such cases, alternative instrument qualification and performance checks should be scientifically justified and documented before use. In addition, validation parameters discussed in this chapter may not be appropriate for all applications of NIR spectroscopy. Validation parameters characterized for a specific NIR-spectroscopic application should demonstrate the suitability of the application for its intended use. For further discussion of the theory and applications of chemometrics, see *Chemometrics* {1039}.

2. QUALIFICATION OF NIR SPECTROMETERS

Qualification of NIR spectrometers is divided into three components: 1) installation qualification (IQ), 2) operational qualification (OQ), and 3) performance qualification (PQ). For further discussion on qualification, see *Analytical Instrument Qualification* {1058}.

2.1 Installation Qualification

The IQ requirements elicit evidence that the hardware and software are properly installed in the desired location.

2.2 Operational Qualification

In OQ, an instrument's performance is characterized using standards to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable. Because there are so many different approaches for measuring NIR spectra, OQ using standards with known spectral properties is recommended. This may be achieved by using the instrument performance checks, and external, traceable certified reference materials (CRMs) as appropriate. As is the case with any spectroscopic device, wavelength accuracy, photometric linearity, and noise characteristics of NIR instruments must be qualified against target specifications for the intended application.

The OQ tests described in the sections that follow are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package. The acceptance specifications given in this section are applicable for general use specifically, whereas specifications for particular instruments and applications can vary, depending on the analytical method used and the desired accuracy of the final result.

2.2.1 CHARACTERIZING INSTRUMENT PERFORMANCE

Use the apparatus according to the manufacturer's instructions, and carry out the prescribed verification at regular intervals. For in-line and on-line applications, the use of alternative means of control of instrument performance must be scientifically justified. For example, use the manufacturer's internal standards or use separate channels/probes, with scientific justification,

to demonstrate instrument performance. System suitability tests may be required prior to sample scanning, and the instrument attributes with the most variation (typically photometric noise and wavelength accuracy) must be tested. The frequency at which each performance test is conducted must be assessed for risk, depending on the instrument type, application, and its environment. For example, instruments placed in harsh environments, such as environments with changing temperature and humidity, will need frequent performance testing.

Wherever possible, in the procedures for determining wavelength accuracy and linearity, analysts must use CRMs rather than laboratory-prepared solutions. These CRMs should be obtained from a recognized, accredited source and should include independently verified, traceable value assignments with associated calculated uncertainty. CRMs are to be kept clean and free from dust. Recertification is to be performed periodically to maintain the validity of the certification.

2.2.2 WAVELENGTH ACCURACY

NIR spectra from sample and/or reference standard materials can be used to demonstrate an instrument's suitable wavelength-dispersion performance against target specifications. Typically, wavelength accuracy is characterized from a single spectrum (collected with the same spectral resolution used to obtain the standard value) using a minimum of three peaks that cover a suitable spectral range of the instrument.

Verification of wavelength scale (except for filter apparatus): Verify the wavelength scale utilized, which is generally in the region between about 780 and about 2500 nm (about 12,800 to about 4000 cm^{-1}) or in the intended spectral range using one or more suitable wavelength standards that have characteristic maxima or minima within the range of methodology wavelengths to be used. Fourier transform (FT) instruments have a linear frequency range; therefore, wavelength certification at a single frequency is sufficient. The USP Near IR System Suitability RS can be used for wavelength verification. Suitable materials for demonstrating wavelength dispersion performance must be used. With appropriate justification, alternative standards may be used. Additional information on calibration considerations may be found in (1856).

Typical tolerances for agreement with standard values are:

- ± 1.0 nm at 780 nm (± 16 cm^{-1} at 12,800 cm^{-1})
- ± 1.0 nm at 1200 nm (± 8 cm^{-1} at 8300 cm^{-1})
- ± 1.0 nm at 1600 nm (± 6 cm^{-1} at 6250 cm^{-1})
- ± 1.5 nm at 2000 nm (± 4 cm^{-1} at 5000 cm^{-1})
- ± 3.0 nm at 2500 nm (± 2 cm^{-1} at 4000 cm^{-1})

For the reference material used, apply the tolerance for the nearest wavelength (or wavenumber) from the above information for each peak used. For diode array instruments, the pixel resolution (wavelength between pixels) can be as large as 10 nm. The pixel resolution must be consistent with the required spectral resolution. The peak-finding algorithms are critical to wavelength accuracy. From a practical standpoint, ± 2 nm is appropriate for peak wavelength accuracy when using such instrumentation. Alternatively, refer to the manufacturer's specifications for acceptance.

2.2.3 PHOTOMETRIC LINEARITY AND RESPONSE STABILITY

Photometric linearity is typically characterized in the range from 10% to 90% reflectance (or transmittance). NIR applications based on measuring an absorbance larger than 1.0 may require standards with reflectivity properties between 2% and 5% reflectance (or transmittance) for characterizing instrument performance at low reflectance. The purpose is to demonstrate a linear relationship between NIR reflectance and/or transmittance and instrument response across the scanning range of the instrument. Subsequent verifications of the photometric model can use the initial observed absorbance values as the established values.

Measure four photometric standards across the working method absorbance range. Typical tolerances for a linear relationship are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept of a plot of the measured photometric response versus the standard photometric response. Alternative tolerances may be used when justified for specific applications. A tolerance of $\pm 2\%$ is acceptable for long-term stability.

2.2.4 PHOTOMETRIC NOISE

NIR instrument software may include built-in procedures to automatically determine system noise and to provide a statistical report of noise or signal-to-noise ratio (S/N) over the instrument's operating range. In addition, it may be desirable to supplement such checks with measurements that do not rely directly on manufacturer-supplied procedures. Typical procedures involve measuring spectra of traceable reference materials with both high and low reflectance. Determine the photometric noise using suitable reflectance standards. Tolerances for these procedures should demonstrate suitable S/N for the intended application. Follow the manufacturer's methodology and specifications.

High-flux noise: Instrument noise is evaluated at high-light flux by measuring reflectance or transmittance of the reference standard, with the reference material (e.g., 99% reflectance standard) acting as both the sample and the background reference. **Low-flux noise:** The same procedure may be used with a lower-reflectivity reference material (e.g., 10% reflectance standard) to determine system noise at reduced-light flux. The source, optics, detector, and electronics make significant contributions to the noise under these conditions.

2.3 Performance Qualification

The objective of PQ is to ensure that the instrument is performing within specified limits with respect to critical operational parameters. In certain cases, when the instrument has been set up for a specific measurement, it might no longer be possible or desirable to measure the wavelength and photometric (signal) qualification reference standards used in the OQ. Provided that the instrument OQ has shown that the equipment is fit for use, a single external performance verification standard can be

used on a continuing basis (e.g., daily or before use). The performance verification standard must match the format of the samples in the current analysis as closely as possible and must use similar spectral acquisition parameters. Quantitative measurements of an external performance verification standard can be used to check both the wavelength accuracy and the photometric precision. Favorable comparison of a series of performance verification spectra demonstrates proper continued operation of the instrument.

Specific procedures, acceptance criteria, and time intervals for characterizing and monitoring NIR instrument performance are selected based on the instrument and intended application. Many NIR applications use previously validated models that relate NIR spectral response to a physical or chemical property of interest. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from sample spectra using previously validated chemometric models.

3. PROCEDURE

NIR spectra can be obtained from several sample presentations, including solids, powders, slurries, gels, liquids, films, and gases. In addition, measurement can be performed through glass or plastic films that are normally used for containment. Typically, NIR spectroscopy does not require any sample preparation because the detectors have a wide dynamic range, and the collection time can be adjusted by the user. Acceptable acquisition parameters or ranges hereof, must be included as a part of the analytical test procedure. Equally important, data pretreatment must be properly implemented such that the intended measurement sensitivity is not subsequently degraded. Specific values for tests such as spectrometer noise and bandwidth will be dependent on the instrument chosen and the purpose required. For this reason, specific instrument tests for these parameters are not indicated here. Additional details can be found in <1856>.

Change to read:

4. VALIDATION AND VERIFICATION

The objective of NIR procedure validation, as is the case with validation of any analytical procedure, is to demonstrate that it is suitable for its intended purpose. NIR spectroscopy is somewhat different from conventional vibrational spectroscopic techniques, because it is not a primary technique. Validation can be achieved by the assessment of variability (precision) and comparison to a reference standard or the result of a validated primary method (accuracy). The procedure for validation is related to the fundamental validation characteristics required for any analytical procedure. Data pretreatment is often a vital step in the chemometric analysis of NIR spectral data. Many suitable data pretreatments exist; the selection should be based on sound scientific judgment and suitability for the intended application.

4.1 Validation

The validation criteria described below are only required when an NIR spectroscopic procedure is intended for use as an alternative to the monograph procedure for testing an official article. The objective of procedure validation is to demonstrate that the measurement is suitable for its intended purpose, including: quantitative determination of the main component in a drug substance or drug product (Category I assays); quantitative determination of impurities or limit tests (Category II); and identification tests (Category IV; see *Validation of Compendial Procedures* <1225>, Table 2). Depending on the category of the test, the analytical procedure validation process for an NIR spectroscopic method requires the testing of accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and robustness as appropriate. Note that validated NIR spectroscopy procedures may not be transferrable to all configurations of NIR spectrometers because of differences in their inherent performance characteristics.

Chapter <1225> provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The following sections are intended to provide the user with specific validation criteria that represent the minimum expectations for this technology, assuming use of the typical Category I USP specifications of 98.0%–102.0% for a drug substance and 90.0%–110.0% for a drug product. The actual validation performance characteristics will depend on the specifications in place and must provide adequate evidence that the measurement capability is sufficient for those specifications. For certain particular applications, tighter criteria may be needed in order to demonstrate suitability for the intended use.

4.1.1 ACCURACY

Accuracy is demonstrated by showing the closeness of agreement between the value that is obtained and either a conventional true value or an accepted reference value from a reference procedure. Accuracy can be determined by direct comparison between validation results and actual or accepted reference values. Suitable agreement with reference values is based on required measurement capability for a specific application. The purpose is to demonstrate a linear relationship between the NIR spectroscopic results and actual values. If a chemometric model is used, accuracy can be determined by using the methodologies in <1039>. The error of the reference procedure may be known 1) on the basis of historical data, 2) through validation results specific to the reference procedure, or 3) by calculating the standard error of the laboratory (SEL).

For Category I and Category II procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. It is also an acceptable practice to compare assay results obtained using the NIR spectroscopy procedure under validation with those obtained from an established alternative analytical method.

Validation criteria

Criteria 1: Suitable agreement between ▲the standard error of prediction (SEP)▲ (ERR 1-May-2020) and SEL is based on required measurement capability for a specific application. For more information, see <1039>.

Criteria 2: In addition to Category I and Category II procedures, 98.0%–102.0% recovery for a drug substance, 95.0%–105.0% recovery for a drug product assay, and 70.0%–150.0% recovery for an impurity analysis. These criteria must be met throughout the intended range.

4.1.2 PRECISION

The precision of an NIR spectroscopic procedure expresses the closeness of agreement among a series of measurements under prescribed conditions. Precision measurements are typically expressed as the relative standard deviation of a series of results and should be suitable for the intended application. Two levels of precision should be considered for NIR spectroscopic procedures: repeatability and intermediate precision.

Repeatability: Repeatability can be demonstrated by statistical evaluation of multiple NIR procedure results, with each result derived from a replicate analysis of a sample after repositioning between spectral acquisitions. System precision may be obtained directly from the analysis of variance (ANOVA) table. The analytical procedure can be assessed by measuring the concentrations of six independently prepared sample preparations at 100% of the assay test concentration. Alternatively, it can be based on measurements of three replicates of three separate samples at different analyte levels. The three levels should be close enough that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations may be pooled for comparison to the acceptance criteria.

Validation criteria: The relative standard deviation is NMT 1.0% for a drug substance assay, NMT 2.0% for a drug product assay, and NMT 20.0% for an impurity analysis.

Intermediate precision: Analysts must test the effects of changes in variables, such as performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts in the repeatability study. At a minimum, any scientifically justified combination of at least two of these factors totaling (based upon intended application) six experiments will provide an estimation of intermediate precision.

Validation criteria: The relative standard deviation is NMT 1.0% for a drug substance assay, NMT 3.0% for a drug product assay, and NMT 25.0% for an impurity analysis.

4.1.3 SPECIFICITY

The extent of specificity testing depends on the intended application. Specificity is typically demonstrated by using the following approaches:

Qualitative: Identification testing is a common application of qualitative NIR spectroscopy. Identification is achieved by comparing a sample spectrum to a reference spectrum or spectra, for instance, by establishing a mathematical relationship (chemometric model). The specificity of the NIR identification procedure is demonstrated by obtaining positive identification from samples, coupled with negative results from materials that should not meet the criteria for positive identification. Materials to demonstrate specificity should be selected on the basis of sound scientific judgment and risk specific to the application (e.g., materials handled in the same area).

Quantitative: Quantitative applications of NIR spectroscopy typically first establish a mathematical relationship (chemometric model) between NIR spectral response and a physical or chemical property of interest. Demonstration of specificity against a physical or chemical property of interest is based on interpreting both NIR spectral attributes and chemometric parameters in terms of the intended application. The demonstration of specificity may include the following:

- Spectral regions can be correlated to the property of interest
- Wavelengths used by regression analysis for the calibration (e.g., for multiple linear regression models) or the loading vector for each factor (e.g., for partial least squares or principal component regression models) can be examined to verify relevant spectroscopic information that is used for the mathematical model
- Variation in spectra from samples for calibration should be examined and interpreted
- Variation in material composition and sample matrix may be shown to have no significant effect on quantification of the property of interest within the specified procedure range

For Category I and Category II procedures, the specificity is demonstrated by meeting the accuracy requirements.

For Category IV tests, the identity of the analyte must be confirmed by comparison with appropriate reference substances.

4.1.4 QUANTITATION LIMIT

Given that NIR methods are usually macro techniques, there is normally no need for determining the quantitation limit. However, fitness for purpose must be demonstrated over the operational range of the analyte.

4.1.5 LINEARITY

Quantitative procedures generally attempt to demonstrate a linear relationship between NIR spectral response function and the property of interest. Although demonstrating a linear response is not required for all NIR applications, the model chosen should properly represent the relationship.

Linearity of NIR spectroscopic procedures depends on variables such as matrix effects and data pretreatment. Validation of linearity in NIR procedures may be accomplished by examining NLT 5 samples that span the operational range and by plotting either the NIR spectral response functions versus actual or accepted values for the property of interest. Many applications may require models of higher order, and various statistical procedures are available for evaluation of the goodness of fit. Applicable statistics and graphical procedures may be used as appropriate.

The Pearson correlation coefficient, r , measures the strength and direction of the association between two variables (x and y), in this instance, concentration and absorbance. The coefficient of determination, r^2 , is a measure of the fraction of the data's variation that is adequately modelled and not a measure of linearity. Linearity depends on the standard error of the calibration function (and hence the reference procedure) and on the range of the calibration data. Thus, although values very near 1.00,

such as 0.99 or greater, typically indicate a linear relationship, lower values do not distinguish between nonlinearity and variability.

Validation criteria: The coefficient of determination, r^2 , must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests. Visual inspection of the residual plots should reveal no significant pattern. For further guidance on multivariate procedures, see <1039>.

4.1.6 RANGE

The specified range of an NIR spectroscopic procedure depends on the specific application. The range typically is established by confirming suitable measurement capability (accuracy and precision) over the proposed operational range. Controls must be used to ensure that results outside of the validated range are not accepted. In certain circumstances, it may not be possible or desirable to extend the validated range to include sample variability outside of the validated range. Extending the range of an NIR spectroscopic procedure requires demonstration of suitable measurement capability within the limits of the expanded range. Examples of situations in which only a limited sample range may be available are samples from a controlled manufacturing process and in-process samples. A limited procedure range does not preclude the use of an NIR spectroscopic procedure.

Validation criteria: For Category I procedures, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For noncentered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the range is 70.0%–130.0%. For Category II procedures, the validation range covers typically 50.0%–120.0% of the acceptance criteria.

4.1.7 ROBUSTNESS

The robustness of an analytical measurement must be demonstrated during development by making deliberate changes to experimental parameters. NIR spectroscopic measurement parameters selected to demonstrate robustness will vary depending on the application and the sample's interface with the instrument. Critical measurement parameters associated with robustness often are identified and characterized during method development. Typical measurement parameters include the following:

- Effect of environmental conditions (e.g., temperature, humidity, and vibration)
- Effect of sample temperature
- Sample handling (e.g., fiber-optic probe depth, compression of material, sample depth/thickness)
- Influence of instrument changes (e.g., lamp change, warm-up time)

4.2 Verification

US Current Good Manufacturing Practices regulations [21 Code of Federal Regulations (CFR) 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of method verification is to demonstrate that the procedure, as prescribed in specific monographs, is being executed with suitable accuracy, sensitivity, and precision. *Verification of Compendial Procedures* <1226> notes that if the verification of the compendial procedure, according to the monograph, is not successful, the procedure may not be suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices*.

Although complete revalidation of a compendial procedure is not required, verification of the compendial NIR spectroscopic procedure includes the determination of certain validation parameters. When the method being verified is for identification purposes, specificity is the only parameter required. For quantitative applications, additional validation parameters, such as accuracy and precision, must be studied as indicated in *Validation of Compendial Procedures* <1225>, *Validation* as appropriate.

USP Reference Standards <11>

USP Near IR System Suitability RS▲ (USP 1-May-2020)