Add the following:

▲⟨858⟩ RAMAN SPECTROSCOPY

INTRODUCTION

Raman is a vibrational spectroscopic technique and is complementary to infrared (IR) and near-infrared (NIR) spectroscopy. The Raman effect itself arises as a result of a change in the polarizability of molecular bonds during a given vibrational mode and is measured as inelastically scattered radiation. The appearance of a Raman spectrum is much like that of an IR absorbance spectrum. The intensities, or the number of Raman photons counted, are plotted against the shifted energies. The Raman shift is usually expressed in wavenumber and represents the energy difference between the incident photon energy and the inelastically scattered photon energy. Unlike IR or NIR spectra that arise when vibrational modes in a molecule have a dipole change, Raman spectra have frequencies and intensities that arise when these same modes cause a change in polarizability. The spectrum is interpreted in the same manner as the corresponding mid-infrared spectrum. The positions of the (Raman-shifted) wavenumbers for a given vibrational mode are theoretically identical to the wavenumbers of the corresponding bands in an IR absorption spectrum. However, the stronger peaks in a Raman spectrum are often weak, or not present in an IR spectrum, and vice versa. Similarly, wavenumbers above 1500 cm^{-1} are group frequencies, and strong bands that absorb below 1500 cm^{-1} can be either group frequencies or fingerprint bands for the molecule. Thus, the two spectroscopic techniques are often said to be complementary. For discussion of the theory and principles of measurements, see ▲*Raman Spectroscopy—Theory and Practice* ⟨1858⟩▲ (CN 1-Aug-2020), which may be a helpful, but not a mandatory, resource. For further discussion of the theory and applications of chemometrics, see *Chemometrics* ⟨1039⟩.

QUALIFICATION OF RAMAN SPECTROMETERS

Qualification of Raman spectrometers is divided into three components: 1) installation qualification (IQ); 2) operational qualification (OQ); and 3) performance qualification (PQ). For further discussion, see *Analytical Instrument Qualification* ⟨1058⟩ and ▲⟨1858⟩▲ (CN 1-Aug-2020).

Installation Qualification

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

Operational Qualification

The requirements for OQ are application and user dependent. Therefore the user needs to specify fitness for purpose requirements for that application and use selection from below as appropriate. For example, applications for establishing the identity of materials may have different requirements than those for quantitative purposes.

Acceptance criteria given in this section are applicable for general use. Acceptance criteria for particular instruments and applications can vary, depending on the analytical method used and the desired accuracy of the final result. American Society for Testing and Materials (ASTM) Standard Reference Materials (SRMs) are also specified, with the understanding that in some circumstances (specifically, remote online applications), calibration using one of these materials may be impractical and other suitably verified materials can be used.

WAVELENGTH ACCURACY

The accuracy of the wavelength axis is ensured via calibration to maintain the integrity of Raman peak positions, see ▲⟨1858⟩▲ (CN 1-Aug-2020). The instrument wavelength accuracy can be determined by using a Raman-shift standard or other suitably high-purity material (e.g., acetaminophen, cyclohexane, or polystyrene). Selection of a standard with bands present across the full Raman spectral range is recommended so that instrument wavelength accuracy can be evaluated at multiple locations within the spectrum. Using a suitable, well-characterized wavenumber standard, the analyst conducts the calibration scans over the spectral range of the analytical system wavenumbers and compares the wavenumber of maximum response of several chosen bands using the appropriate peak-picking algorithms to the known Raman shift of the standard (see *Table 1*). Instrumentation for quantitative purposes must perform to these requirements. For qualitative requirements, the maximum allowable acceptance

criteria for all wavelengths contained in *Table 1* is ±3.0 cm^{-1}. Under these circumstances the user is required to demonstrate fitness for purpose of the derived calibration model over the operational range.

Table 1. Wavenumber Shifts of Polystyrene, Acetaminophen (paracetamol), and Cyclohexane with Acceptance Criteria[a]

Wavenumber Shifts[b] (cm^{-1})		Acceptance Criteria (cm^{-1})
	620.9	±1.5
	1001.4	±1.5
	1031.8	±1.5
	1602.3	±1.5
Polystyrene[c]	3054.3	±3.0
	797.2	±1.5
	857.9	±1.5
	1168.5	±1.5
	1236.8	±1.5
	1323.9	±1.5
	1648.4	±1.5
Acetaminophen[d]	2931.1	±2.0
	801.3	±1.5
	1028.3	±1.0
	1266.4	±1.0
	1444.4	±1.0
Cyclohexane[e]	2852.9	±2.0

[a] Range testing during wavelength accuracy can be adjusted to the application for which instrument is being used. For instance, if the method does not use wavelengths above 2900 cm^{-1}, the wavelength accuracy test may only consider reference peaks below that value.
[b] *Standard Guide for Raman Shift Standards for Spectrometer Calibration* (ASTM E1840).
[c] Polystyrene foil (30 mil, 76 µm) or polystyrene pellets [National Institute of Standards and Technology (NIST) 706a], or Certified Reference Materials (CRMs), traceable to NIST SRMs, or other international or national standards, are commercially available and should be used where possible.
[d] Acetaminophen for equipment qualification CRS.
[e] Cyclohexane reference material.

[NOTE—For scanning dispersive instruments, calibration might need to be performed more frequently, and precision in both a scanning and static operation mode may need to be verified.]

PHOTOMETRIC PRECISION

Laser variation in terms of the total emitted photons occurring between two measurements can give rise to changes in the photometric precision of the instrument. Unfortunately, it is very difficult to separate changes in the photometric response associated with variations in the total emitted laser photons from the sample and sampling-induced perturbations. This is one of the reasons why the photometric precision acceptance criteria are set relatively loosely; a maximum tolerance of 10% from reference measurements made from the reference material is applied.

Performance Qualification

The objective of PQ is to ensure that the instrument is performing within specified limits with respect to wavelength accuracy and photometric precision. 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 instrument OQ has shown that the system is fit for use, a single external performance verification standard may 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.

PROCEDURE

Raman 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, Raman spectroscopy does not require any sample preparation because it is relatively insensitive to physical state solid or emulsion, slurry, etc. Furthermore, Raman uses back scattering geometry, right angle geometry, or transmission geometry, so the frequency and intensity are not affected much. Any intensity difference may arise from sample non-homogeneity and sampling area difference. The detectors have a wide dynamic range, and the collection time can be adjusted by the user.

Considerations about exposure of the sample to the laser must be taken into account because high-intensity lasers and small measurement spots may result in burning of the sample.

Acceptable acquisition parameters and a summary of the validation results must be included as part of the respective analytical test procedure. Specific values for tests will depend on the instrument chosen and the required purpose. For this reason, specific instrument tests for these parameters are not indicated here. For additional details, see ▲⟨1858⟩▲ (CN 1-Aug-2020), which provides a detailed discussion of Raman spectroscopy.

VALIDATION AND VERIFICATION

The objective of Raman procedure validation, as is the case with validation of any analytical process, is to demonstrate that the measurement is suitable for its intended purpose. 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 Raman spectral data. Many suitable data pretreatments exist; the selection should be based on sound scientific judgment and suitability for the intended application. See ⟨1039⟩ and *Mid-Infrared Spectroscopy* ⟨854⟩.

Validation

The validation criteria described below are only required when a Raman procedure is intended for use as an alternative to the official procedure for testing an official article. The objective of a Raman procedure validation is to demonstrate that the measurement is suitable for its intended purpose, including the following: quantitative determination of the main component in a drug substance or a 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 process for analytical procedure validation for Raman spectroscopy may require the testing of accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and robustness. Validated Raman procedures may not be transferable to all models and configurations of Raman spectrometers because of differences in their inherent performance characteristics.

⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The sections that follow are intended to provide the user with specific validation criteria that represent the minimum expectations for this technology, assuming the typical Category I USP acceptance criteria of 98.0%–102.0% for a drug substance and 90.0%–110.0% for a drug product. The actual validation performance characteristics would depend on the acceptance criteria in place and must provide sufficient evidence that the measurement capability is sufficient for these acceptance criteria. For each particular application, tighter criteria may be needed to demonstrate suitability for the intended use.

ACCURACY

For Category I and II procedures, accuracy can be determined by conducting recovery studies with prepared samples or appropriate reference materials over the operational concentration range taking into account matrix effects. Comparison of assay results obtained using the Raman procedure under validation with those obtained from an established alternative analytical procedure may also be used to determine accuracy. See ⟨1039⟩ for more information.

Validation criteria: 98.0%–102.0% recovery for drug substances, 95.0%–105.0% recovery for drug product assays, and 70.0%–150.0% recovery for impurity analyses. These criteria should be met throughout the intended range.

PRECISION

Repeatability: The analytical procedure can be assessed by measuring the concentrations of six independently prepared sample preparations at 100% of the assay test concentration. Alternatively, this assessment can be based on measurements of three replicates of three separate samples at different concentrations. The three concentrations should be close enough that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

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

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

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

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 Raman spectroscopy. Identification is achieved by comparing a sample spectrum to a reference spectrum or spectra either visually or by, for instance, establishing a "goodness of fit" criterion or use of a chemometric model. The specificity of the Raman 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 Raman spectroscopy typically first establish a mathematical relationship (chemometric model) between Raman 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 Raman 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 should be shown to have no significant effect on quantification of the property of interest within the specified procedure range as part of robustness studies

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.

QUANTITATION LIMIT

Given that Raman methods are typically multivariate, there is no need for determining a defined quantitation limit. However, fitness for purpose is demonstrated over the operational range of the analyte.

LINEARITY AND CALIBRATION MODELS

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

Linearity of Raman spectroscopic procedures depends on variables such as matrix effects and data pretreatment. Validation of linearity in Raman procedures may be accomplished by examining NLT 5 samples that span the expected concentration range and by plotting either the Raman spectral responses 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 modeled 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: Visual inspection of the residual plots should reveal no significant pattern. For further guidance on multivariate procedures, see ⟨1039⟩.

RANGE

The specified range of a Raman 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 a Raman 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 a Raman spectroscopic procedure.

Validation criteria: For Category I procedures, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For non-centered 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 typically covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The robustness of an analytical measurement must be demonstrated during development by making deliberate changes to experimental parameters. For Raman spectroscopy, this can include, but is not limited to, changes in sample position, laser–sample interaction, or hardware settings. See ▲ ⟨1858⟩▲ (CN 1-Aug-2020) for additional factors impacting measurement.

Verification

United States Current Good Manufacturing Practice regulations [21 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, the users must verify suitability under actual conditions of use.

The objective of a Raman spectroscopic procedure 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 and Requirements, 6.30 Alternative and Harmonized Methods and Procedures*.

Although complete revalidation of a compendial procedure is not required, verification of the compendial Raman spectroscopic procedure includes the demonstration of certain validation parameters. When the procedure being verified is for identification purposes, specificity is the only parameter required. However, for quantitative applications, additional validation parameters must be studied as appropriate; these may include accuracy and precision over the expected concentration range.

▲ (USP 1-Aug-2020)