Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-May-2020
Document Type: General Chapter
DocId: GUID-21493947-8F57-4FC7-9338-FD47C1354A3A_4_en-US
DOI: https://doi.org/10.31003/USPNF_M3208_04_01
DOI Ref: ocg6x

⟨854⟩ MID-INFRARED SPECTROSCOPY

INTRODUCTION

Mid-infrared (mid-IR) spectroscopy is an instrumental method used to measure the absorption of electromagnetic radiation over the wavenumber range between 4000 and 400 cm^{-1} (corresponding to wavelengths between 2.5 and 25 µm). Unless otherwise specified in a monograph or other validated procedure, the region from 3800 to 650 cm^{-1} (corresponding to wavelengths from 2.6 to 15 µm) should be used to ensure compliance with monograph specifications for IR absorption. The absorption of photons causes the promotion of molecules from a ground state of their vibrational mode to an excited vibrational state.

Vibrational modes are defined by the motion of all atoms in a molecule. When molecules contain certain functional groups, IR absorption often occurs in specific narrow spectral ranges. In these cases, the wavenumbers at which these transitions occur are known as group frequencies. When a vibrational mode involves atomic motions of more than just a few atoms, the frequencies occur over wider spectral ranges and are not characteristic of a particular functional group but are more characteristic of the molecules as a whole. Such bands are known as fingerprint bands. All strong bands that absorb at wavenumbers above 1500 cm^{-1} are group frequencies. Strong bands that absorb below 1500 cm^{-1} can be either group frequencies or fingerprint bands.

For discussion of the theory and principles of measurements, see *Mid-Infrared Spectroscopy—Theory and Practice* ⟨1854⟩, which may be a helpful, but not mandatory, resource.

QUALIFICATION OF IR SPECTROPHOTOMETERS

Qualification of mid-IR spectrophotometers is divided into three components: *Installation Qualification* (IQ), *Operational Qualification* (OQ), and *Performance Qualification* (PQ). For further information, see *Analytical Instrument Qualification* ⟨1058⟩.

Installation Qualification

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

Operational Qualification

Because the majority of mid-IR spectra are measured with Fourier-transform IR (FTIR) spectrophotometers, only these instruments will be discussed. [Note—No recommended values for signal-to-noise ratio or 100% line stability are included in this chapter because these vary with manufacturer, model, and age of the instrument.]

WAVENUMBER ACCURACY

The most commonly used wavenumber standard for IR spectrophotometry is an approximately 35 µm thick, matte polystyrene film. The spectrum of such a film has several sharp bands at 3060.0, 2849.5, 1942.9, 1601.2, 1583.0, 1154.5, and 1028.3 cm^{-1}. The most frequently chosen band for wavenumber accuracy determination is located at 1601.2 cm^{-1}. Using a suitable polystyrene film or other well-characterized wavenumber standard, scan from 3800 to 650 cm^{-1} wavenumbers, and compare the wavenumber of maximum response of the chosen band using the center-of-gravity, polynomial spline procedure, or other peak-picking algorithms to the known absorption wavenumber of the standard. The acceptable tolerance for the measured wavenumber is ±1.0 cm^{-1}.

Performance Qualification

The purpose of performance qualification (PQ) is to determine that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement.

PROCEDURE

Mid-IR spectra can be measured by transmission, external reflection, internal reflection (often called attenuated total reflection), diffuse reflection, and photoacoustic spectroscopy. Different sample preparation techniques are available for these options. The most common sample preparation techniques are presented below.

Potassium Bromide (KBr) Disks

Certain powdered alkali halides such as potassium bromide, potassium chloride, and caesium iodide coalesce under high pressure and can be formed into self-supporting disks that are transparent to mid-IR radiation. The alkali halide most commonly used is powdered, dry, highly pure potassium bromide, which is transparent to mid-IR radiation above 400 cm^{-1}.

Commercial presses and dies in a range of diameters are available for the preparation of alkali halide and similar disks.

Mineral Oil Mulls

A typical procedure to prepare a mull is to place 10–20 mg of the sample into an agate mortar, and then grind the sample to a fine particle-size powder using a vigorous rotary motion of the pestle. A small drop of the mulling agent is added to the mortar. Rotary motion of the pestle is used to mix the components into a uniform paste, which is transferred to the center of a clean IR-transparent window (e.g., potassium bromide, sodium chloride, silver bromide, or caesium iodide). A second matching window is placed on top of the mull, and the mull is squeezed to form a thin, translucent film that is free from bubbles.

The most widely used mulling agent for the mid-IR region is a saturated hydrocarbon mineral oil (liquid paraffin, Nujol).

Self-Supported Polymer Films

The mid-IR transmission spectrum of many polymers used as packaging materials is at times recorded from samples prepared as thin self-supporting films using hot compression molding or microtoming.

Capillary Films

Nonvolatile liquids can be examined neat in the form of a thin layer sandwiched between two matching windows that are transparent to mid-IR radiation. The liquid layer must be free of bubbles and must completely cover the diameter of the IR beam focused on the sample.

Liquids and Solutions in Transmission Cells

For the examination of liquid and solution samples, transmission cell assemblies that comprise a window pair, spacer, filling ports, and a holder are available commercially in both macro- and micro-sample configurations.

For laboratory applications, spacers typically are formed from lead, poly(tetrafluoroethylene), or poly(ethylene terephthalate) and can be supplied, depending on spacer materials, in standard thickness path lengths from approximately 6 µm to 1 mm or larger.

Gases

Mid-IR transmission cells for static or flow-through gas and vapor sampling are available in a wide range of materials to suit the application, from laboratory to process scale. In the laboratory, the traditional gas cell has been a 10 cm long cylinder made from borosilicate glass or stainless steel with an approximately 40-mm aperture at each end. Each open end is covered with an end cap that contains one of a pair of mid-IR–transparent windows constructed from, e.g., potassium bromide, zinc selenium, or calcium fluoride.

Attenuated Total Reflection

Attenuated total reflectance spectroscopy relies on the optical phenomenon of radiation passing through a medium of high refractive index at a certain angle of incidence entirely reflected internally at a boundary in contact with a material of lower refractive index. The medium of high refractive index is also known as the internal reflection element (IRE).

The sample under examination should be placed in close contact with the IRE such as diamond, germanium, zinc selenide, or another suitable material of high refractive index. Ensure close and uniform contact between the substance and the whole crystal surface by applying pressure in the case of solid samples or by dissolving the substance in an appropriate solvent and then covering the IRE with the solution and evaporating to dryness.

Diffuse Reflection

The most important and commonly used form of sample preparation for diffuse reflection is to dilute the sample by intimately mixing it with 90%–99% of nonabsorbing diluents such as finely powdered potassium bromide or potassium chloride. The sample dilution has the added benefit of reducing absorption band intensities to an appropriate level.

Microscope Sampling

Coupling a light microscope with a mid-IR spectrophotometer allows spectra to be obtained from very small samples. Generally applied in transmittance or reflectance modes, it provides, for example, a powerful tool for obtaining spectroscopic data of contaminants in pharmaceutical samples.

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VALIDATION AND VERIFICATION

Validation

Validation is required when an IR method is intended for use as an alternative to the official procedure for testing an official article.

The objective of an IR method 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 a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV, see *Table 2* in *Validation of Compendial Procedures* ⟨1225⟩). Depending on the category of the test, the validation process for IR may require the testing of linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness. If the IR procedure employs a chemometrics model calculated against the response of another analytical technology (e.g., HPLC), then the principles of ▲*Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩▲ (CN 1-May-2020), specifically the *Method Validation* section, is to be applied.

Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation for each characteristic. The following sections are intended to provide the user with specific validation criteria that represent the minimum

expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

ACCURACY

For Category I and 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 IR procedure under validation with those obtained from an established alternative analytical method.

Validation criteria: 98.0%–102.0% mean recovery for drug substances, 95.0%–105.0% mean recovery for drug product assay, and 70.0%–150.0% mean recovery for impurity analysis. These criteria are met throughout the intended range.

PRECISION

Repeatability: The analytical procedure is 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 sample solutions at different concentrations. The three concentrations should be close enough so 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 drug substance, NMT 2.0% for drug product, and NMT 20.0% for impurity analysis.

Intermediate precision: The effect on analytical precision caused by changes in variables such as performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts needs to be assessed. At a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

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

SPECIFICITY

For Category IV tests, the identity of the analyte should be ensured. Regarding Category I and II procedures, the accuracy requirement also demonstrates specificity for the targeted analytes.

In the case of identification tests, the ability to select between compounds of closely related structure that are likely to be present should be demonstrated. This should be confirmed by obtaining positive results (perhaps by comparison to a known reference material) from samples containing the analyte, coupled with negative results from samples that do not contain the analyte and by confirming that a positive response is not obtained from materials structurally similar to or closely related to the analyte.

QUANTITATION LIMIT

The quantitation limit can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank preparation divided by the slope of the calibration line and multiplying by 10. Other suitable approaches can be used (see ⟨1225⟩). A measurement of a representative sample matrix spiked at the estimated quantitation limit concentration must be performed to confirm accuracy.

Validation criteria: For the estimated quantitation limit to be considered valid, the measured concentration must be accurate and precise at a level equal to or less than 50% of the specification.

LINEARITY

A linear relationship between the analyte concentration and the IR spectral response is demonstrated by preparing NLT 5 standard preparations at concentrations encompassing the anticipated concentration of the test preparation. The standard curve should then be evaluated using appropriate statistical methods such as a least squares regression. For experiments that do not have a linear relationship between analyte concentration and IR spectral response, appropriate statistical methods should be applied to describe the analytical response.

Validation criteria: Correlation coefficient (R), NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests.

RANGE

This parameter is demonstrated by meeting linearity, precision, and accuracy requirements.

Validation criteria: For Category I tests, 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 validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters. For mid-IR this can include but is not limited to changes in sample preparation procedure or changes in hardware settings.

Verification

U.S. Current Good Manufacturing Practices 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, they must simply verify their suitability under actual conditions of use.

The objective of an IR procedure verification is to demonstrate that the method, 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 is not suitable for use with the article under test. It may be necessary to develop and validate an alternative procedure as allowed in *General Notices, 6.30 Alternative and Harmonized Methods and Procedures* .

Although complete revalidation of a compendial procedure is not required, verification of the compendial Mid-IR procedure includes the execution of certain critical parameters. When the method being verified is for identification purposes, specificity is the only parameter required. For quantitative applications, additional validation parameters are studied. Typically these include accuracy, precision, and quantitation limit, as indicated in *Validation*.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<854> MID-INFRARED SPECTROSCOPY	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

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Pharmacopeial Forum: Volume No. PF 40(1)

Current DocID: GUID-21493947-8F57-4FC7-9338-FD47C1354A3A_4_en-US
DOI: https://doi.org/10.31003/USPNF_M3208_04_01
DOI ref: ocg6x