⟨197⟩ SPECTROSCOPIC IDENTIFICATION TESTS

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INTRODUCTION AND SCOPE

This chapter provides several tests and procedures that are used to confirm the chemical identity of a specific material in its respective monograph. When one of the tests listed in this chapter is referenced in a monograph, it must be used to confirm the identity of the material. Alternative identification procedures may be used, provided they are demonstrated to be equivalent to or better than the specified procedure and if they meet the requirements specified in the *Equivalent/Alternative Tests* section.

Spectroscopic tests contribute meaningfully toward the identification of many official articles. The test procedures that follow are applicable to substances that absorb, transmit, reflect, or scatter electromagnetic radiation in the near-infrared (NIR), infrared (IR), visible or near-infrared (Raman), ultraviolet (UV), visible (Vis), or X-ray ranges (see *Mid-Infrared Spectroscopy* ⟨854⟩, *Ultraviolet-Visible Spectroscopy* ⟨857⟩, *X-Ray Powder Diffraction* ⟨941⟩, *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩, and ▲*Raman Spectroscopy* ⟨858⟩▲ (CN 1-Aug-2020). The NIR, IR, and Raman spectra, or X-ray diffraction pattern of a substance, compared with the spectrum or diffraction pattern obtained with the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test. The UV or Vis absorption spectrum of a substance, on the other hand, does not exhibit a high degree of specificity in most cases. To provide unambiguous confirmation of the identity of a substance, it may be necessary to execute two (or more) identity tests, as specified in a large proportion of compendial monographs.

IDENTIFICATION METHODOLOGY

Suitable identification methodology must be used for the chemical identification of materials through comparison with the appropriate compendial standards. Where alternative techniques are used, it must be demonstrated that the alternative identification methodology is suitable for the intended application (see *Validation of Compendial Procedures* ⟨1225⟩). Identification procedures should be able to discriminate between materials similar in molecular structure. The lack of specificity of a single technique may be compensated by other supporting analytical procedure(s) or an application of an additional identification technique.

The IR spectrum of a substance, compared with that obtained using equivalent instruments and conditions for the corresponding USP Reference Standard, is the most widely used methodology for chemical identification in compendial monographs. In general practice, the analysis of the sample and the USP Reference Standard are completed at the same time; however, if the USP Reference Standard spectrum was obtained previously using equivalent instruments and conditions, it is appropriate to compare the sample spectrum with the stored USP Reference Standard spectrum.

Under conditions where the IR spectrum lacks specificity for definitive chemical identification, additional spectroscopic information can be used to supplement chemical identification. For example, conformance with both IR and UV test specifications, as specified in a large proportion of compendial monographs, provides complementary information for the definitive identity of the sample under examination. In these instances, the combined spectroscopic information enables discrimination between compounds similar in structure that would not be possible from either IR spectrum or UV spectrum alone.

For preparing the standards used in the applications of this chapter, unless the directions for preparing the USP Reference Standard are explicitly specified in the monograph procedure, the USP Reference Standard must be used in accordance with instructions on its label. For preparation of the test sample, the sample must be prepared in accordance with the directions in the individual monograph procedure. Where no specific instructions are provided for sample preparation, handle the sample in the same manner as described by the USP Reference Standard label. For example, dry the sample as per drying conditions on the label of the corresponding USP Reference Standard.

INFRARED SPECTROSCOPY

Several methods are indicated for the preparation of test samples and USP Reference Standards for analysis by infrared spectroscopy (see *Table 1* and ⟨854⟩). The approaches for the techniques used in IR identity testing are summarized in *Table 1*.

Table 1. Infrared Spectroscopy Sample Preparation Techniques[a]

Method Reference	Sample Preparation
⟨197A⟩	The substance under examination is intimately in contact with an internal reflection element for attenuated total reflection (ATR) analysis.
⟨197D⟩	The substance under examination is mixed intimately with potassium bromide and transferred to a sample container for diffuse reflection (DR) analysis.
⟨197E⟩	The substance under examination is pressed as a thin sample against a suitable plate for IR microscopic analysis.
⟨197F⟩	The substance under examination is a thin film of a neat liquid or semisolid between suitable (e.g., sodium chloride or potassium bromide) plates, or a thin microcrystalline or glassy film deposited from a solution or cooled from a melt.
⟨197K⟩	The substance under examination is mixed intimately with potassium bromide and compressed into a transparent pellet.

Table 1. Infrared Spectroscopy Sample Preparation Techniques[a] *(continued)*

Method Reference	Sample Preparation
⟨197M⟩	The substance under examination (10–20 mg) is finely ground and dispersed in a drop of mulling agent (unless otherwise directed in the monograph, mineral oil is to be used).
⟨197S⟩	A solution of designated concentration is prepared in the solvent specified in the individual monograph. The solution is examined in 0.1-mm cells, unless a different cell path length is specified.

[a] Each of the techniques in the table can be used as alternative methods when any other technique from the table is required in the monograph (see *General Notices, 6.30 Alternative and Harmonized Methods and Procedures*).

In each instance, infrared spectra of both the sample and corresponding USP Reference Standard are obtained using the same sample preparation technique and measurement parameters. Record and compare the spectra of the sample and the corresponding USP Reference Standard over the range from 3800 to 650 cm^{-1}, unless otherwise specified in the individual monograph. The comparison must establish that the IR spectrum of the preparation of the sample exhibits maxima only at the same wavenumbers as that of the appropriately prepared corresponding USP Reference Standard. If there are differences between the spectra, and the sample spectrum was compared with a previously obtained and electronically stored spectrum of the USP Reference Standard, the comparison must be repeated concomitantly with a freshly prepared USP Reference Standard.

Differences between the USP Reference Standard spectrum and sample spectrum that may be observed are sometimes attributable to differences in the solid-state form of the materials, if a solid-state technique is used (e.g., ⟨197A⟩, ⟨197K⟩, or ⟨197M⟩). If a specific crystal form is not specified in the monograph, where spectral differences between the sample and USP Reference Standard are observed, recrystallize both the sample and USP Reference Standard under identical conditions to produce the same solid-state form, unless specific procedures are provided in the individual monographs. Dissolve equal portions of the sample and the USP Reference Standard in equal volumes of a suitable solvent, evaporate the solutions to dryness in similar containers under identical conditions, and repeat the identification test on the residues. Other techniques for recrystallizing the sample and USP Reference Standard based on known scientific principles may be used with appropriate scientific justification.

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NEAR-INFRARED AND RAMAN SPECTROSCOPY

The reference ⟨197NIR⟩ in a monograph signifies that a test sample and a standard sample are examined by the NIR spectroscopic technique (see *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩), and the reference ⟨197R⟩ in a monograph signifies that a test sample and a standard sample are examined by the Raman spectroscopic technique (see ▲⟨858⟩▲ (CN 1-Aug-2020)). NIR and Raman spectroscopic procedures may be used to confirm the identity of materials. The approach is similar to that of IR identity testing but is often augmented through the use of spectral libraries and chemometrics. A sample can thus be analyzed by NIR or Raman techniques, with the resulting spectrum compared with stored spectra in the spectral library through the use of multivariate analysis. In general, a visual comparison or simple overlay of the spectra alone may not be sufficient and additional evaluation may be needed.

In both techniques, samples can be directly interrogated with minimal or no sample preparation. Measurement is nondestructive and noninvasive, and data collection can often be made through glass or plastic containers.

A description of these techniques, and the strategy for procedure and chemometrics model development and validation can be found in the appropriate associated chapters when available (see *Chemometrics* ⟨1039⟩, *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩, and ▲*Raman Spectroscopy—Theory and Practice* ⟨1858⟩▲ (CN 1-Aug-2020), and the following chapters to be published at a later date: *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩ and *Raman Spectroscopy—Theory and Practice* ⟨1858⟩).

ULTRAVIOLET-VISIBLE SPECTROSCOPY

The reference ⟨197U⟩ in a monograph signifies that a sample solution and a Standard solution are examined spectroscopically, in 1-cm cells, over the spectral range from 200 to 400 nm, unless otherwise specified in the individual monograph (see ⟨857⟩). Dissolve a portion of the substance under examination in the designated medium to obtain a sample solution having the concentration specified in the monograph. Record and compare the spectra obtained for the sample solution and the Standard solution. Review or calculate the absorptivities and/or absorbance ratios and compare the results, and where appropriate, compare to criteria specified in an individual monograph.

The comparison must establish that the UV spectrum of the preparation of the sample exhibits absorption maxima and minima only at the same wavelengths as those of the appropriately prepared corresponding USP Reference Standard, and that the absorptivities and/or absorbance ratios are within the specified limits. If there are differences in the spectra, and the sample spectrum was compared with a previously obtained and electronically stored spectrum of the USP Reference Standard, the comparison must be concomitantly repeated with a freshly prepared USP Reference Standard.

Unless otherwise specified in the monograph, absorbances indicated for the calculations of the absorptivities and/or absorbance ratios are those measured at the maximum absorbance wavelength (within ±2 nm) specified in the individual monograph. Where the absorbance is to be measured at about the specified wavelength other than that of maximum absorbance, the abbreviations for minimum (min) and shoulder (sh) are used, respectively, in an absorption spectrum.

The reference ⟨197U-LC⟩ in a monograph signifies that, when a diode array detector is used in tandem with an LC procedure test in the monograph, the spectra of the major chromatographic peak(s) of the sample solution and Standard solution are examined spectroscopically.

The requirements are met if the UV spectra of the sample solution and of the Standard solution exhibit maxima and minima at the same wavelengths, and, if applicable, the absorptivities and/or absorbance ratios are within specified limits.

X-RAY POWDER DIFFRACTION

The reference ⟨197XR⟩ in a monograph signifies that a test sample and a standard sample are examined according to ⟨941⟩.

Prepare and mount the specimen as directed in ⟨941⟩. Unless otherwise indicated in the monograph, record the diffraction pattern in a 2θ-range from as near to 0° as possible to at least 32°. Unless otherwise specified in the monograph, the requirements are met if the X-ray diffraction pattern of the test specimen conforms to that of the corresponding USP Reference Standard obtained using equivalent instruments and conditions. Differences in the diffraction line intensities (but not line positions) between the sample and the Standard are acceptable.

The comparison must establish that the diffraction pattern of the preparation of the test specimen conforms to the diffraction pattern of the corresponding USP Reference Standard. If there are differences in the diffraction pattern and the sample diffraction pattern was compared with a previously obtained and electronically stored diffraction pattern of the USP Reference Standard, the comparison must be repeated concomitantly with a freshly prepared USP Reference Standard.

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EQUIVALENT/ALTERNATIVE TESTS

In addition to IR, NIR, Raman, X-ray, and UV absorption, several other spectroscopic methodologies can be utilized for the identification of the specimen under examination. The methods cited in this chapter may be used for identification of materials as an alternative method to the method referenced in the monograph, provided that the alternative technique has been determined to be suitable for identification. Suitable identification tests must be able to discriminate between compounds similar in molecular structure that are likely to be present. The choice of such potentially interfering materials must be based on sound scientific judgment, with consideration of interferences that could occur. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination).

For information regarding sample preparation and measurement parameters associated with an alternative identification method, refer to the appropriate general chapter (see *Mass Spectrometry* ⟨736⟩, *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩, ⟨854⟩, ⟨857⟩, ⟨941⟩, *Near-Infrared Spectroscopy—Theory and Practice* ⟨1856⟩, and ▲ ⟨858⟩▲ (CN 1-Aug-2020).