

The Establishment of Performance Verification Procedures for Fourier Transform Infrared Spectrometers

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Abstract

An integral part of any laboratory Quality Management System (QMS) is the ability to verify an analytical instrument's performance prior to analyzing official samples. Performance verification procedures provide the instrument user the means to assess whether a system is functioning properly. They are a routine predetermined set of tests and acceptance criteria that can be provided by the instrument manufacturer and/or established by the laboratory itself. Performance verification procedures that have been established for a Fourier transform infrared (FT-IR) microscope system outfitted with a macro sampling accessory allow the user to determine the daily and/or annual functionality of the instrument. Specifications were established for the IR microscope operating in transmission, reflection/absorption (R/A) and attenuated total reflection (ATR) sampling modes. The specifications for the macro sampling accessory were established for transmission and ATR sampling modes only. The acceptance criterion for each performance verification specification was calculated from a series of measurements made using each accessory and sampling mode. The performance verification procedures, as well as their associated specification and acceptance criteria described in this study, are general enough that they can be applied to FT-IR instruments from different manufacturers.

Keywords:

Spectrometer, Infrared spectroscopy, Infrared microscope, Performance verification procedures, Standard operating procedures

1. Introduction

Before a new instrument can be used for official sample work, a Standard Operating Procedure (SOP) must be written and approved. The equipment SOP typically includes performance verification procedures, which provide the instrument user the means to assess whether a system is functioning properly. In the case of infrared spectrometers, the instrument's performance verification procedures usually include evaluating:(a) the energy (optical throughput), which assesses the amount of light reaching the detector;(b)the qualitative short term drift, which assesses the short term stability of the instrument;(c) the peak-to-peak noise, which assesses the sensitivity of the instrument; and, (d) the wavelength accuracy, which compares measured peak positions to known peak positions of a polystyrene reference standard. Although manufacturers often have software packages that include automatic performance verification procedures, the procedures may have limitations: they may be time-consuming; they may not include all of the important performance specifications; and, they may not meet the require-

ments of the laboratory's accreditation and/or QMS requirements. Further, acceptance criteria are not always established for the instrumental conditions most commonly employed by the laboratory. In one case acceptance criteria were only established for a microscope using a room-temperature triglycine sulfate (TGS) detector, which is typically not employed for microspectroscopic analyses in which small sample areas significantly limit the optical throughput. Instead, liquid nitrogen-cooled mercury cadmium telluride (MCT) detectors are employed for such analyses due to their much greater sensitivity. Due to limitations encountered with many automatic performance verification procedures, the FCC typically establishes and executes performance standards for each of its infrared spectrometers. These standards are in accordance with guidelines set forth by the American Society for Testing and Materials (ASTM) [1] and the European Pharmacopoeia (European Pharmacopoeia) [2], and are outlined in the SOP for each infrared spectrometer [3–7].

1.1. Background

Infrared spectroscopy and microspectroscopy are employed on a routine basis for acquiring molecular information on a wide variety of materials such as bulk powders, tablet coatings,

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tablet cores, capsule shells, capsule contents, dietary supplements, adhesives, and packaging components. For a more detailed explanation of the evolution of the technique and a fundamental description of the instrumentation see references [8, 9].

A variety of different sampling modes can be used for macroscopic and microscopic infrared analysis, and include transmission, R/A and ATR. A diagram of each sampling mode has been provided in Figure 1. (micro configuration only) and a detailed description of each sampling mode can be found elsewhere [9]. A brief explanation will be provided for all three modes to illustrate the need to establish specific performance verification procedures for each.

In a transmission modality (Figure. 1a), light is received from underneath the microscope optics and directed toward the condenser by an input mirror. The condensing optics focus the light onto the sample. Light diverging from the sample is received and directed toward the detector by the objective. This approach is ideal for slightly absorbing materials such as thin films or flattened fibers but problematic for strongly absorbing, thick samples. In a reflection/absorption method (Figure. 1b), the condensing optics are not used. Instead, light is directed toward roughly half of the objective by an input mirror positioned above the objective. Light converging from the objective is focused onto a sample that is placed on top of a reflective substrate, such as a low-E (SnO_2 coated) microscope slide or a gold-coated metal plate. The light penetrates the sample at an angle, θ , reflects off of the substrate and then emerges from the sample. The diverging light is received and directed toward the detector by the other half of the objective. This technique is useful for analyzing films that are too thin for, or not compatible with transmission analysis such as a film residue dried down on a microscope slide after being extracted with a solvent from a complex mixture.

Internal reflection methods (Figure. 1c) have recently become the standard sampling technique for many infrared analyses because they are very useful for most sample types, including highly absorbing materials. In the case of a microscope, light is directed into an ATR objective by an input mirror positioned above the objective. Light converging from the objective is passed through the internal reflection element (IRE) and focused onto its surface, which is in contact with the sample, at an angle greater than the critical angle of the IRE. As the light internally reflects off of the IRE surface, the evanescent wave penetrates a defined distance into the sample (typically 1–5 μm) and is then reflected back through the IRE. Light diverging from the IRE is received and directed toward the detector by the other half of the ATR objective. Due to the well-defined evanescent wave volume, internal reflection methods are sample-thickness independent (assuming the sample thickness is greater than the depth of penetration of the evanescent wave). The technique is often capable of accommodating the as-received state of the sample (little or no sample preparation) and is useful for unknown powders, tablet coatings, tablet cores, packaging products and many other materials.

2. Experimental

2.1. Instrumental Conditions

All micro and macro spectroscopic data were collected on a Thermo iN10 FT-IR microscope and a Thermo iZ10 FT-IR macro accessory, respectively. All micro data were collected on a single day. All macro data were collected on a single day two days after the micro data. Data were acquired using the procedure outlined in FCC SOP E154 [3] and with the parameters listed in Table 1. The instrument includes a potassium bromide (KBr) beam splitter, which is ideal for use in IR instruments for two reasons. The material transmits light over a large region of the mid-infrared spectrum, and it provides an optimal tradeoff between reflected and transmitted light. A room-temperature TGS detector was suitable for the macro analyses due to the larger sample area, and thus larger optical throughput characteristic of this approach. The micro spectroscopic analyses required the more sensitive MCT detector because small sample sizes significantly restricted the amount of light reaching the detector. Spectral resolution of 4 cm^{-1} was employed because the natural line widths of most condensed phased materials are greater than 4 cm^{-1} . The upper and lower limits of the spectral range, or the region of the infrared spectrum over which data are collected, are determined by the beam splitter, source, instrument optics and/or detector. The macro measurements were conducted between 4000 – 600 cm^{-1} , which is the standard range used for an ATR macro accessory with a TGS detector. The upper limit was chosen because few fundamental mid-infrared vibrations are observed above 4000 cm^{-1} and the lower limit was dictated by the cut-off of the TGS detector. The micro measurements were conducted between 4000 – 675 cm^{-1} . The upper limit was chosen for the same reason discussed with the TGS detector and the lower limit was dictated by the cut-off of the MCT detector. The number of coadditions (n) required a tradeoff between data collection time and signal-to-noise ratio (SNR) since SNR is directly proportional to \sqrt{n} . The instrument manufacturer recommended an average of 64 scans, which takes one minute to theoretically achieve an 8-fold increase in SNR compared to a single scan. Griffiths et al. recommended the use of Norton-Beer medium or Happ-Genzel apodization functions for most applications that may or may not require high quantitative accuracy [8]. Happ-Genzel was chosen for the current study in order to stay consistent with FCC library spectra. The gain, phase correction, optical velocity, aperture, zero filling and filters were all set to the default setting(s) recommended by the manufacturer (for more information on these parameters refer to reference [8]).

2.2. Materials

For the wavelength accuracy test each sampling mode required a unique polystyrene secondary reference standard because the interaction between the light and sample was different for each method. For example, the transmission standard consisted of a thin film, the R/A standard consisted of a thin film mounted on a gold coated plate and the ATR standard consisted of a much thicker piece of polystyrene. In many cases polystyrene standards are received with the instrument and have

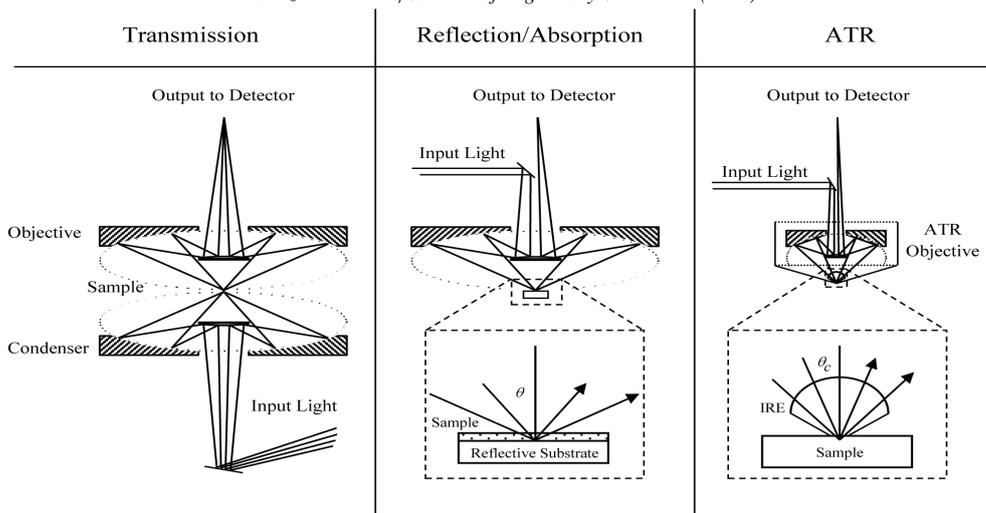


Figure 1. Infrared sampling methods.

Table 1. Instrumental parameters.

	Beam Splitter	Detector	Spectral Resolution (cm^{-1})	Spectral Range (cm^{-1})	Scans	Apodization
Macro						
Transmission	KBr	TGS	4	4000-600	64	Happ-Genzel
ATR	KBr	TGS	4	4000-600	64	Happ-Genzel
Micro						
Transmission	KBr	MCT	4	4000-675	64	Happ-Genzel
Reflectance	KBr	MCT	4	4000-675	64	Happ-Genzel
ATR	KBr	MCT	4	4000-675	64	Happ-Genzel

been measured against certified standards using the company's International Standards Organization (ISO) 9001 compliant procedure. If reference standards were not received with the instrument, they were obtained externally. All certified standards are traceable to samples measured at the National Institute of Standards and Technology (NIST) and have been reported in the NIST Certificate for Standard Reference material 1921a.

3. Discussion

After following the steps outlined in FCC SOP E154 [3] acceptance criteria were established for each sampling mode of the FT-IR microscope and macro sampling accessory. Three background, noise (open beam/no sample in the sample compartment) and sample spectra (polystyrene reference standard) were collected for each sampling mode. Performance verification was measured with regard to a) energy (optical throughput), b) qualitative short term drift (instrument stability), c) peak-to-peak noise (sensitivity) and d) wavelength accuracy (as compared to a reference standard). The acceptance criteria are provided in Appendix 1 and were established using the following guidelines.

3.1. Acceptance Criteria: Energy

Representative background spectra for each sampling mode are shown in Figure 2. The strong, narrow peaks between $4000 - 3400 \text{ cm}^{-1}$ and between $2100 - 1300 \text{ cm}^{-1}$ are attributed to rotational absorptions of atmospheric water vapor. The strong, broad peaks between $2400 - 2300 \text{ cm}^{-1}$ (Figures. 2a-2e) and the narrow peak at 668 cm^{-1} (Figures. 2a-2b) are correlated to the O-C-O antisymmetric stretching vibration and the O-C-O bending vibration of atmospheric carbon dioxide, respectively. The latter band was not observed in the micro spectra because the low wavenumber cutoff of the MCT detector was higher than the location of this absorption. The intensity of atmospheric water vapor and carbon dioxide bands will vary over time and these regions should be avoided when establishing performance specifications and associated acceptance criteria.

Unique features in the macro ATR background spectrum (Figure. 2b) between $2700 - 2400 \text{ cm}^{-1}$ and between $2300 - 1800 \text{ cm}^{-1}$ were attributed to absorption by the diamond IRE. The micro ATR accessory is made out of germanium, which does not absorb IR energy in this region. The average maximum single-beam intensity (observed around 1900 cm^{-1} for each sampling mode) has been recorded in Appendix 1. The energy acceptance criterion was established by multiplying each

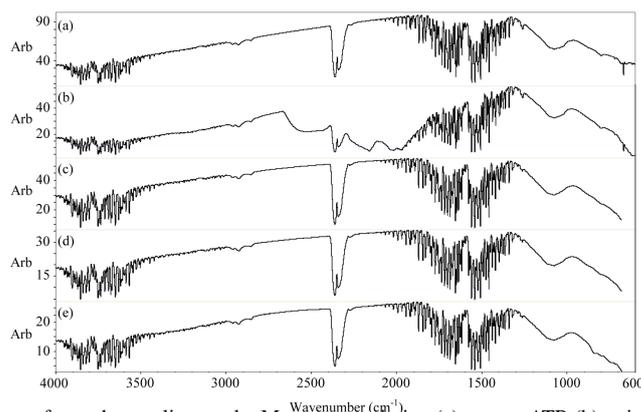


Figure 2. Representative background spectra for each sampling mode. Macro transmission (a), macro ATR (b), micro transmission (c), micro reflection/absorption (d) and micro ATR (e).

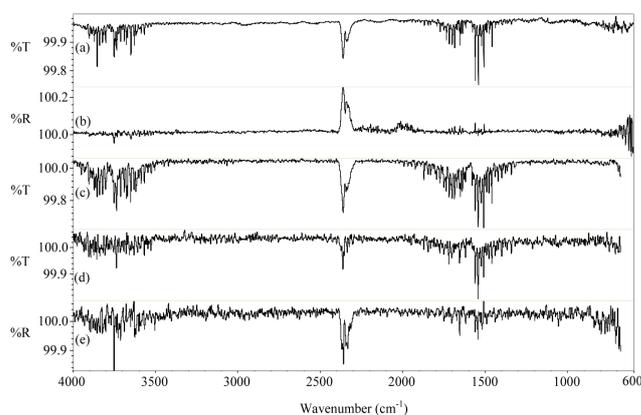


Figure 3. Representative noise spectra for each sampling mode. Macro transmission (a), macro ATR (b), micro transmission (c), micro reflection/absorption (d) and micro ATR (e).

value by 80%, which takes into account source intensity and detector response fluctuations. The macro transmission maximum single-beam intensity was the greatest of all modalities because nearly all the incident light reached the detector. The macro ATR mode intensity was approximately half of the transmission mode intensity because of losses due to absorption by the IRE. The micro transmission intensity was also approximately half that of the macro transmission intensity. However, this decrease was the result of using a smaller aperture, which limited the amount of light reaching the detector (optical throughput). The intensities for the micro R/A and ATR modalities were even less because only half of the objective was used by these reflective techniques (see Figure. 1 and corresponding discussion). The intensity of the micro ATR technique was further diminished because of absorption by the IRE.

3.2. Acceptance Criteria: Qualitative Short Term Drift

Representative noise spectra for each sampling mode are shown in Figure. 3. The predetermined acceptance criterion for qualitative short term drift required all data points in the noise spectrum to be between 99 – 101% T[1], which was observed for each sampling method. There were, however, spectral regions that contained signals greater than the instrumental noise. For example, the larger noise between 2300 – 1800 cm^{-1} in the macro ATR spectrum is due to absorption by the diamond IRE. Rotational vibrations of water vapor and O-C-O antisym-

metric stretching vibrations of carbon dioxide are due to constantly changing atmospheric conditions. For higher sensitivity measurements, the sampling accessories can be purged with a non-absorbing infrared gas such as N_2 . However, this purging process is not often required.

Noise spectra with downward bands (Figures. 3a, 3c, 3d and 3e) indicated a higher concentration of atmospheric carbon dioxide and water vapor compared to the time the background was taken. On the other hand, the noise spectrum with inverted bands (Figure. 3b) indicated a lower concentration of these atmospheric gases at the time the noise spectrum was taken. The macro and micro ATR noise spectra exhibit a significantly decreased level of water vapor compared to the other sampling modes due to the much shorter distance (pathlength) that the infrared light is exposed to the atmosphere with these approaches (μm compared to mm with transmission and R/A).

3.3. Acceptance Criteria: Peak-to-Peak Noise

The average and standard deviation peak-to-peak noise values were measured and recorded for each sampling mode between 2600 – 2400 cm^{-1} because this region was free of interference from atmospheric water vapor, carbon dioxide and absorption by the IRE (Appendix 1). The peak-to-peak noise acceptance criterion was established by multiplying the standard deviation by a factor of ten and adding this value to the average peak-to-peak noise value.

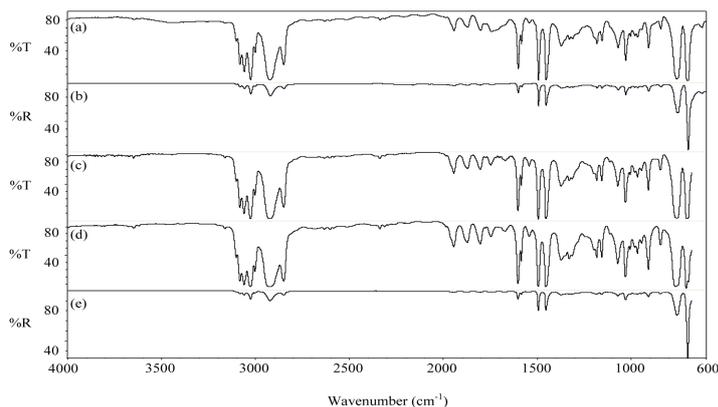


Figure 4. Representative polystyrene spectra for each sampling mode. Macro transmission (a), macro ATR (b), micro transmission (c), micro reflection/absorption (d) and micro ATR (e).

Table 2. Infrared band assignments for polystyrene.

Assignment	Peak Position (cm ⁻¹)
Aromatic C-H Stretch	3100-3000
CH ₂ Antisymmetric Stretch	2823
CH ₂ Symmetric Stretch	2850
Quadrant Ring Stretch	1601
Semicircle Ring Stretch	1493
Semicircle Ring Stretch/CH ₂ symmetric Scissor Deformation	1453
Ring In-Phase CH Bend	1029
Monosubstituted Ring In-Phase CH Wag	757
Monosubstituted Ring Out-of-Plane Bend	700

3.4. Acceptance Criteria: Wavelength Accuracy

The reference wavenumber positions were obtained from the certification documents that were received with the polystyrene reference standards. Three reference bands (one from the high, medium and low-wavenumber region) were recorded in Appendix 1 for each sampling mode for comparison to the wavenumber positions measured empirically. Not all sampling modes used the same three bands because the manufacturer used different peaks to calibrate each polystyrene reference material. Representative polystyrene spectra for each sampling mode are shown in Figure 4. Some characteristic vibrations have been listed in Table 2 [10]. The measured wavenumber positions were compared to the three reference wavenumber positions and were recorded in Appendix 1 for each sampling mode. The acceptance criterion for wavelength accuracy required all measured peaks to be ± 1 cm⁻¹ with regard to the reference peaks [2]. If necessary, this criterion can be increased to ± 2 cm⁻¹ to account for slight shifting that is observed using ATR. The transmission spectra (Figures. 4a and 4c) and reflectance spectrum (Figure. 4d) are nearly identical and the ATR spectra are nearly identical because of similarities between the sampling methods. Although all spectra share the same bands, there are several intensity differences between the ATR spectra and the transmission and R/A spectra. For example, the 2924, 1493, 1452, 757 and 700 cm⁻¹ band intensities are nearly the same for the transmission and reflectance spectra. In both ATR spectra the 700 cm⁻¹ band is at least twice the intensity of the other bands (and the 757 cm⁻¹ band is much larger than the 1493 and 1452 cm⁻¹ bands and these bands are larger than the 2924 cm⁻¹

band). The intensity differences are the result of the ATR process. Specifically, the depth of penetration of the evanescent beam increases with decreased wavenumber, which results in a smaller sample volume (less intense absorptions) in the high wavenumber region and a larger sample volume (more intense absorptions) in the low wavenumber region. Sample spectra can be normalized for pathlength (ATR correction feature in the Omnic software) to provide spectra that are more consistent with a transmission or R/A spectrum.

3.5. Daily Performance Verification Tests

The performance verification specifications and acceptance criteria established for this FT-IR system form the basis for a daily performance verification procedure. The tests used on a daily basis are sensitive enough to detect instrumental problems, which may have a negative impact on sample work. The daily performance verification procedure is outlined in FCC SOP E154 [3] and the corresponding daily log sheet has been provided in Appendix 2. Each day prior to use, a background, noise and polystyrene spectrum will be collected (and saved) using the parameters listed in Table 1. These spectra will be examined to determine the instrument energy, short term drift, peak-to-peak noise and wavelength accuracy relative to the acceptance criteria, which will be taken from the values described above. Once the three spectra have been acquired, the measured values will be recorded and the appropriate line in Appendix 2 will be initialed. The instrument will have to pass all performance verification criteria every day prior to use of the spectrometer. However, the test will only need to be performed

for the sampling mode(s) that will be used for a given day. For example, only the macro ATR performance verification will be completed if this is the only sampling mode used for that day.

4. Conclusion

This study described a procedure for establishing performance verification specifications and acceptance criteria for an FT-IR microscope operating in transmission, R/A and ATR sampling modes and an FT-IR macro sampling accessory operating in transmission and ATR sampling modes. Using this data, the study also described a simple daily quality assurance verification test to monitor the instrument's performance. Although the acceptance criteria in Appendices 1 and 2 are applicable to the microscope and macro sampling accessory described in this study, these procedures can be used to establish performance verification procedures for any FT-IR instrument.

5. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest. Research was funded by U.S. Food and Drug Administration.

6. Disclaimer

The views expressed are those of the authors and should not be construed to represent the views or policies of the U.S. Food and Drug Administration. Any reference to a specific commercial product, manufacturer, or otherwise, is for the information and convenience of the public and does not constitute an endorsement, recommendation or favoring by the U.S. Food and Drug Administration.

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7. Article information

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