



Standard Practice for Determining Relative Spectral Correction Factors for Emission Signal of Fluorescence Spectrometers¹

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1. Scope

1.1 This practice (1)² describes three methods for determining the relative spectral correction factors for grating-based fluorescence spectrometers in the ultraviolet-visible spectral range. These methods are intended for instruments with a 0°/90° transmitting sample geometry. Each method uses different types of transfer standards, including 1) a calibrated light source (CS), 2) a calibrated detector (CD) and a calibrated diffuse reflector (CR), and 3) certified reference materials (CRMs). The wavelength region covered by the different methods ranges from 250 to 830 nm with some methods having a broader range than others. Extending these methods to the near infrared (NIR) beyond 830 nm will be discussed briefly, where appropriate. These methods were designed for scanning fluorescence spectrometers with a single channel detector, but can also be used with a multichannel detector, such as a diode array or a CCD.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*³

[E131 Terminology Relating to Molecular Spectroscopy](#)

[E388 Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers](#)

¹ This practice is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and Separation Science and is the direct responsibility of Subcommittee E13.01 on Ultra-Violet, Visible, and Luminescence Spectroscopy.

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² The boldface numbers in parentheses refer to a list of references at the end of this standard.

³ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[E578 Test Method for Linearity of Fluorescence Measuring Systems](#)

[E2719 Guide for Fluorescence—Instrument Calibration and Qualification](#)

3. Significance and Use (Intro)

3.1 Calibration of the responsivity of the detection system for emission (EM) as a function of EM wavelength (λ_{EM}), also referred to as spectral correction of emission, is necessary for successful quantification when intensity ratios at different EM wavelengths are being compared or when the true shape or peak maximum position of an EM spectrum needs to be known. Such calibration methods are given here and summarized in [Table 1](#). This type of calibration is necessary because the spectral responsivity of a detection system can change significantly over its useful wavelength range (see [Fig. 1](#)). It is highly recommended that the wavelength accuracy (see [Test Method E388](#)) and the linear range of the detection system (see [Guide E2719](#) and [Test Method E578](#)) be determined before spectral calibration is performed and that appropriate steps are taken to insure that all measured intensities during this calibration are within the linear range. For example, when using wide slit widths in the monochromators, attenuators may be needed to attenuate the excitation beam or emission, thereby, decreasing the fluorescence intensity at the detector. Also note that when using an EM polarizer, the spectral correction for emission is dependent on the polarizer setting. (2) It is important to use the same instrument settings for all of the calibration procedures mentioned here, as well as for subsequent sample measurements.

3.2 When using CCD or diode array detectors with a spectrometer for λ_{EM} selection, the spectral correction factors are dependent on the grating position of the spectrometer. Therefore, the spectral correction profile versus λ_{EM} must be determined separately for each grating position used. (3)

3.3 Instrument manufacturers often provide an automated procedure and calculation for a spectral correction function for emission, or they may supply a correction that was determined at the factory. This correction can often be applied during spectral collection or as a post-collection correction. The user

TABLE 1 Summary of Methods for Determining Spectral Correction of Detection System Responsivity

NOTE 1—“Drop-In” refers to whether or not the material/hardware can be put in the sample holder and used like a conventional sample; “Off-Shelf” refers to whether or not the material/hardware can be purchased in an immediately-usable format; “Uncertainty” is the estimated expanded (k=2) total uncertainty; “Caveats” refer to important information that a user should know about the method before attempting to use it; “Certified Values” refers to whether or not the material/hardware is supplied with appropriate values as a function of emission wavelength and their corresponding total uncertainties; the references (Ref.) give examples and more in-depth information for each method.

Method	λ_{EM}	Drop-In	Off-Shelf	Uncertainty	Caveats	Certified Values	Ref.
CS	UV-NIR	N	Y	$< \pm 5 \%$	difficult setup	Y	E578, (3-6)
CD+CR	UV-NIR	N	Maybe	$\pm 10 \%$	difficult setup	Y	E578, (4, 5, 7)
CRMs	UV-NIR	Y	Y	$\pm 5 \%$		Y	E131, (8-13)

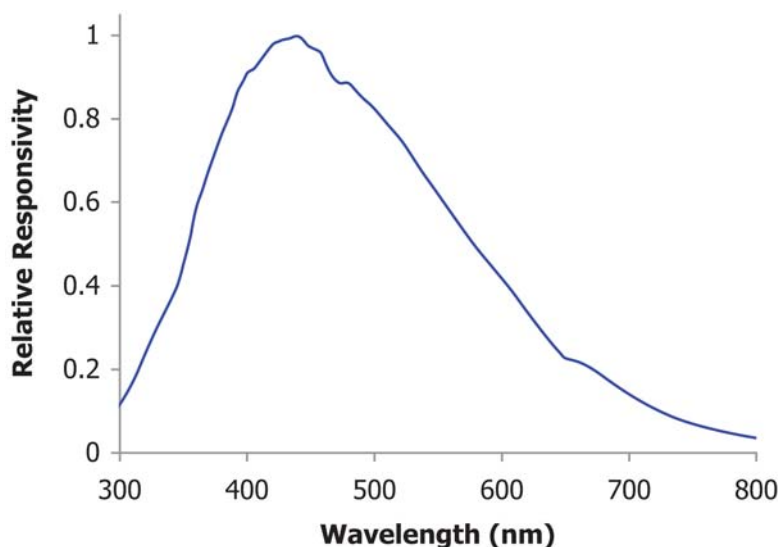


FIG. 1 Example of Relative Spectral Responsivity of Emission Detection System (Grating Monochromator-PMT Based), (see Test Method E578) for which a Correction Needs to be Applied to a Measured Instrument-Specific Emission Spectrum to Obtain its True Spectral Shape (Relative Intensities).

should be advised to verify that the automated vendor procedure and calculation or supplied correction are performed and determined according to the guidelines given within this standard.

4. Calibrated Optical Radiation Source (CS) Method (see Test Method E578, (4-6, 14))

4.1 Materials:

4.1.1 A calibrated tungsten lamp is most commonly used as a CS in the visible region due to its high intensity and broad, featureless spectral profile. Its intensity falls off quickly in the ultraviolet (UV) region, but it can typically be used down to 350 nm or so. It also displays a high intensity in the near infrared, peaking at about 1000 nm. Its intensity gradually decreases beyond 1000 nm, but continues to have significant intensity out to about 2500 nm. A calibrated deuterium lamp can be used to extend farther into the UV with an effective range from about 200 to 380 nm. The effective range of a CS is dependent on the intensity of the CS and the sensitivity of the detection system. This range can be determined by measuring the low-signal regions where the signal profile of the light from the CS becomes flat or indistinguishable from the background signal, implying that the signal afforded by the CS is not measurable in these λ_{EM} regions.

4.1.2 A calibrated reflector (CR) is often used to reflect the light from the CS into the emission detection system. A diffuse reflector made of compressed or sintered polytetrafluoroethylene (PTFE) is most commonly used as a CR, due to its nearly Lambertian reflectance, which prevents both polarization and spatial dependence of the reflectance. In addition, PTFE possesses a reflectance profile that is nearly flat, changing by less than 10 % from 250 to 2500 nm. For a CS and a CR, “calibrated” implies that the spectral radiance and the spectral reflectance, respectively, are known (calibrated wavelength dependence of the spectral radiant factor including measurement uncertainty) and traceable to the SI (International System of Units). This is commonly done through certification of these values by a national metrology institute (NMI). (15, 16, 7)

4.2 Procedure:

4.2.1 Direct the optical radiation from a CS into the EM detection system by placing the CS at the sample position. If the CS is too large to be placed at the sample position, place a CR at the sample position to reflect the optical radiation from the CS into the EM detection system. Ensure that the CS is aligned such that its light is centered on the entrance slit of the λ_{EM} selector, and on all optics it encounters before the entrance slit. Ideally, the light should fully and uniformly fill the