



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

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

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.