Designation: E388 – 04

Standard Test Method for Wavelength Accuracy of Spectral Bandwidth of Fluorescence Spectrometers[1]

This standard is issued under the fixed designation E388; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the testing of the spectral bandwidth and wavelength accuracy of fluorescence spectrometers that use a monochromator for emission wavelength selection and photomultiplier tube detection. This test method can be applied to instruments that use multi-element detectors, such as diode arrays, but results must be interpreted carefully. This test method uses atomic lines between 250 nm and 1000 nm.

1.2 *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. Summary of Test Method

2.1 The difference between the apparent wavelength and the known wavelength for a series of atomic emission lines is used as a test for wavelength accuracy. The apparent width of some of these lines is used as a test for spectral bandwidth.

3. Apparatus

3.1 *Fluorescence Spectrometer* to be tested.

3.2 *Atomic Discharge Lamps, Low-pressure*, sufficiently small to be placed in the sample cell holder of the instrument.

4. Reagent

4.1 *Scattering Suspension*—Dissolve 1 g of glycogen per litre of water, or use a dilute microsphere suspension containing 1 mL of a commercially available, concentrated microsphere suspension.

5. Procedure

5.1 The emission lines given for Hg, Ne, Ar, Kr, and Xe in Table 1 are typically observable using standard commercial fluorometers, although some of them may be too weak to detect on some instruments.

5.1.1 Most fluorescence instruments will not be able to resolve very closely spaced lines such as those for Hg at 312.57 nm, 313.15 nm, and 313.18 nm, due to the relatively low resolution monochromators used in fluorescence equipment compared to those used in absorbance spectrometers. Even lower resolution fluorometers may not resolve lines separated by less than several nanometres such as those for Hg at 404.66 and 407.78, or at 576.96 and 579.07 nm.

5.1.2 In instruments using blazed grating monochromators, additional weaker lines are found due to second order diffraction of atomic lines. For instance, lines appear for mercury at 507.30 and 593.46 nm, arising from the 253.65 and 296.73 nm lines, respectively.

5.2 *Calibration and Adjustment of Emission Monochromator*:

5.2.1 With an atomic arc source properly aligned (see section 5.3) in the sample cell compartment, adjust the position of the wavelength dial to give maximum signal for each of the atomic lines and record the wavelength reading. The difference between the observed value and the corresponding value in Table 1 represents the correction that must be subtracted algebraically from the wavelength reading of the instrument. The corrections may be recorded or the monochromator adjusted to give the proper values. Since there may be some backlash in the wavelength drive of scanning instruments, always approach the peak position from the same direction, if applicable.

5.2.2 When calibrating scanning-type instruments, approach the peak position in the same direction that the motor scans, if your instrument does not correct for backlash. Check the position against that recorded while scanning and, if necessary, correct as in 5.2.1.

5.3 In cases where the monochromator is designed so that a lateral displacement of the calibration source from a position directly in front of the entrance slit appears as a wavelength shift, proceed as follows:

5.3.1 Instead of placing the atomic lamp in front of the entrance slit of the monochromator, fill a sample cell with a dilute scattering suspension, as described in section 4.1.

5.3.2 Place the cell in the sample position in the instrument.

5.3.3 Illuminate the cell transversely with the atomic lamp, either from the side or from above.

[1] This test method is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and Chromatography and is the direct responsibility of Subcommittee E13.01 on Ultra-Violet, Visible, and Luminescence Spectroscopy.

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