Designation: E 388 – 72 (Reapproved 1998)

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

This standard is issued under the fixed designation E 388; 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.

2. Referenced Documents

2.1 *ASTM Standards:*

E 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near Infrared Spectrophotometers[2]

3. Summary of Test Method

3.1 The difference between the apparent wavelength and the known wavelength for a series of mercury 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.

4. Apparatus

4.1 *Fluorescence Spectrometer* to be tested.

4.2 *Mercury Arc, Low-pressure*[3], sufficiently small to be placed in the sample cell holder of the instrument.

5. Reagent

5.1 *Glycogen Suspension*—Dissolve 1 g of glycogen per litre of water, or use a Ludox[4] suspension containing 1 mL of Ludox per litre of water.

6. Procedure

6.1 Lines suitable for calibration are the nine mercury lines at 253.65, 296.73, 334.15, 404.66, 407.78, 435.84, 546.07, 576.96, and 579.07 nm. These are listed in the Mercury Arc Emission Spectrum in the Ultraviolet and Visible Regions fig. of Practice E 275.

6.1.1 Other lines are suitable for calibration such as some of the weaker lines included in the Mercury Arc Emission Spectrum in the Ultraviolet and Visible Regions fig. of Practice E 275, but not included in the above list. The comparatively low resolution monochromators often used in fluorescence equipment may not resolve pairs of lines such as at 404.66 and 407.78, or at 576.96 and 579.07 nm.

6.1.2 In instruments using grating monochromators, additional weaker lines are found due to second order diffraction of mercury lines. Thus, lines appear at 507.30, 593.46, 668.30 nm, arising from the 253.65, 296.73, and 334.15-nm lines, respectively.

6.2 *Calibration and Adjustment of Emission Monochromator*:

6.2.1 With the mercury arc source properly aligned in the sample cell compartment, adjust the position of the wavelength dial to give maximum signal for each of the mercury lines and record the wavelength reading. The difference between the observed value and the corresponding value in 6.1 represents the correction that must be subtracted algebraically from the reading on the dial. The corrections may be recorded or the monochromator adjusted to give the proper values. Since there is some backlash in the wavelength drive, always adjust the dial to the peak reading from the same direction.

6.2.2 When calibrating scanning-type instruments, turn the dial to give the peak reading in the same direction that the dial is turned by the scan motor. Check the dial reading against the value recorded while scanning and, if necessary, correct as in 6.2.1.

6.3 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:

6.3.1 Instead of placing the mercury lamp in front of the entrance slit of the monochromator, fill a sample cell with a Ludox suspension, diluted to 1000 mL with distilled water, or with a glycogen suspension containing 1 g of glycogen per litre of distilled water.

6.3.2 Place the cell in the regular position in the instrument.

6.3.3 Illuminate the cell transversely with the mercury lamp, either from the side or from above.

6.3.4 Adjust the position of the wavelength dial to give the maximum signal for each of the mercury lines in 6.1.1; record the wavelength reading and proceed as in 6.2.

---

[1] This test method is under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.06 on Molecular Luminescence.

Current edition approved June 29, 1972. Published September 1972. Originally published as E 388 – 69 T. Last previous edition E 388 – 69 T.

[2] *Annual Book of ASTM Standards*, Vol 14.01.

[3] The Pen Ray Quartz Lamp, manufactured by Ultraviolet Products, Inc., San Gabriel, CA, and available from apparatus distributors, has been found satisfactory.

[4] Ludox is an aqueous suspension of colloidal silica, manufactured by E. I. DuPont De Nemours and Co., Industrial and Biochemicals Dept., Wilmington, DE.

6.4 *Adjustment of Excitation Monochromator*:

6.4.1 After the emission monochromator has been calibrated, adjust the excitation monochromator to match.

6.4.2 Place a sample cell containing either the Ludox or glycogen suspension in the sample cell compartment to serve as a light scatterer.

6.4.3 With a continuous source (for example, a xenon arc) in the normal operating position of the instrument, illuminate the suspension being used as a light scatterer.

6.4.4 Set the wavelength dials of both excitation and emission monochromators at a previously determined setting used for calibration of the emission monochromator.

6.4.5 Adjust the position of the wavelength dial of the excitation monochromator to give a maximum signal, and record the wavelength reading. The difference between the observed value on the dial and the corresponding value in 6.1 represents the correction that must be subtracted algebraically from the reading on the dial. The corrections may be either recorded, or the monochromator may be adjusted to give the proper value. As stipulated in 6.2.1, always turn the dial to give the peak reading in the same direction that the dial is turned by the scan motor.

6.4.6 The match of the excitation monochromator with the emission monochromator may be checked at wavelengths above or below that used in 6.4.5.

6.5 *Slit Width Effects*:

6.5.1 Use the narrowest practical slit widths in calibrating the wavelength scale. In cases when monochromator slits are not filled, or when intensity of fluorescence varies rapidly with wavelength, there may be an apparent wavelength error with wide slits. Under the most unfavorable conditions this error may approach one spectral bandwidth, so that narrow slits should be used for accurate wavelength measurements. As the magnitude of the error may depend on characteristics of both the instrument and the sample, a generally applicable correction for slit widths is not practical.

6.5.2 For greatest accuracy at important wavelengths of specific compounds, measure the peak wavelength as a function of slit width and plot a correction curve.

6.6 *Spectral Bandwidth of Monochromator*:

6.6.1 Use the well-separated lines such as those at 253.65, 334.15, 435.84, and 546.07 nm. Do not use second order lines.

6.6.2 Take sets of readings at wavelengths on both sides of the reading of maximum intensity.

6.6.3 Record the readings of the wavelength dial that correspond to photometer readings of 50 % and 5 % of the maximum. The wavelength interval at the 50 % points is the spectral bandwidth of the monochromator. This test is used as an indication of the approximate resolving power that may be expected from the instrument. The wavelength interval at the 5 % points is an indication of the degree of isolation that may be achieved between adjacent wavelengths.

7. Keywords

7.1 fluorescence spectrometers; molecular luminescence; molecular spectroscopy