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

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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:

[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.

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[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.

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