~~Designation:E 388–72 (Reapproved 1998)~~ Designation: E388 – 04 (Reapproved 2009)

Standard Test Method for ~~Spectral Bandwidth~~Wavelength Accuracy and ~~Wavelength Accuracy~~Spectral Bandwidth of Fluorescence Spectrometers[1,2]

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

~~1.1This test method covers the testing of the spectral bandwidth and wavelength accuracy of fluorescence spectrometers.~~

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 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:*~~

~~E275Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near Infrared Spectrophotometers~~

~~3.~~Summary of Test Method

~~3.1The~~2.1 The difference between the apparent wavelength and the known wavelength for a series of ~~mercury~~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.

~~4.~~3. Apparatus

~~4.1~~

3.1 *Fluorescence Spectrometer* to be tested.

~~4.2*Mercury Arc, Low-pressure*~~

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

~~5.~~

4. Reagent

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

~~6.~~

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.

~~[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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[1] This test method 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] 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* ~~, Vol 14.01.~~volume information, refer to the standard's Document Summary page on the ASTM website.

5. Procedure

~~6.1Lines 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 275E 275.~~

~~6.1.1Other 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 275E 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.~~

~~6.1.2In 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~~

5.1 The emission lines given for mercury (Hg), neon (Ne), argon (Ar), krypton (Kr), and xenon (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 Hg 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*:

~~6.2.1~~5.2.1 With ~~the mercury~~an atomic arc source properly aligned (see 5.3) in the sample cell compartment, adjust the position of the wavelength dial to give maximum signal for each of the ~~mercury~~atomic lines and record the wavelength reading. The difference between the observed value and the corresponding value in ~~6.1~~Table 1 represents the correction that must be subtracted algebraically from the wavelength reading ~~on~~of the ~~dial.~~instrument. The corrections may be recorded or the monochromator adjusted to give the proper values. Since there ~~is~~ may be some backlash in the wavelength ~~drive, always adjust the dial to~~drive of scanning instruments, always approach the peak ~~reading~~position from the same direction, if applicable.

~~6~~5.2.2 When calibrating scanning-type instruments, ~~turn~~approach the ~~dial to give the~~ peak ~~reading~~position in the same direction that the ~~dial is turned by the scan motor.~~ motor scans, if your instrument does not correct for backlash. Check the ~~dial reading~~ position against ~~the value~~ that recorded while scanning and, if necessary, correct as in ~~6.2.1~~5.2.1.

~~6.3In~~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:

~~6~~5.3.1 Instead of placing the ~~mercury~~atomic lamp in front of the entrance slit of the monochromator, fill a sample cell with a ~~Ludox~~ dilute scattering suspension, ~~diluted to 1000 mL with distilled water, or with a glycogen suspension containing 1 g of glycogen per litre of distilled water.~~ as described in 4.1.

TABLE 1 Atomic Emission Lines[A] for Wavelength Accuracy

Hg	Ne			Ar	Kr		Xe
253.65	336.99	633.44	830.03	696.54	427.40	645.63	450.10
296.73	341.79	638.30	836.57	706.72	428.30	722.41	458.28
302.15	345.42	640.11	837.76	727.29	431.96	758.74	462.43
312.57	346.66	640.22	841.72	738.40	436.26	760.15	467.12
313.15	347.26	650.65	841.84	750.39	437.61	768.53	469.70
313.18	350.12	653.29	846.34	751.47	440.00	769.45	473.42
334.15	352.05	659.90	857.14	763.51	442.52	785.48	480.70
365.02	359.35	667.83	859.13	772.38	445.39	805.95	482.97
404.66	533.08	671.70	863.46	794.82	446.37	810.44	484.33
407.78	534.11	692.95	864.70	800.62	450.24	811.29	491.65
435.84	540.06	703.24	865.44	801.48	557.03	819.00	492.32
546.07	576.44	717.39	865.55	810.37	564.96	826.32	711.96
576.96	582.01	724.52	867.95	811.53	567.25	828.10	764.20
579.07	585.25	743.89	868.19	826.45	583.29	829.81	823.16
	588.19	783.91	870.41	840.82	587.09	850.89	828.01
	594.48	792.71	877.17	842.46	599.39	877.67	834.68
	597.55	793.70	878.06	912.30	601.21	975.18	840.92
	603.00	794.32	885.39	922.45	605.61		881.94
	607.43	808.25	920.18	965.78			895.22
	609.62	811.85	930.09				979.97
	614.31	812.89	932.65				992.32
	616.36	813.64	942.54				
	621.73	825.94	948.67				
	626.65	826.61	953.42				
	630.48	826.71					

[A] Wavelength values have been obtained from Harrison, G.R., MIT Wavelength Tables, *Wavelengths by Element*, Vol 2, MIT Press, Cambridge, MA, 1982; and Zaidel, A.N., Prokofev, V.K., Raiskii, S.M., Slavnyi, V.A., and Shreider, E.Ya., *Tables of Spectral Lines*, Plenum Press, New York, NY, 1970.