Designation: E1247 – 12

Standard Practice for Detecting Fluorescence in Object-Color Specimens by Spectrophotometry[1]

This standard is issued under the fixed designation E1247; 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 practice provides spectrophotometric methods for detecting the presence of fluorescence in object-color specimens.

NOTE 1—Since the addition of fluorescing agents (colorants, whitening agents, etc.) is often intentional by the manufacturer of a material, information on the presence or absence of fluorescent properties in a specimen may often be obtained from the maker of the material.

1.2 This practice requires the use of a spectrophotometer that both irradiates the specimen over the wavelength range from 340 to 700 nm and allows the spectral distribution of illumination on the specimen to be altered as desired.

1.3 Within the above limitations, this practice is general in scope rather than specific as to instrument or material.

1.4 *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:*[2]

D2244 Practice for Calculation of Color Tolerances and Color Differences from Instrumentally Measured Color Coordinates

E284 Terminology of Appearance

E308 Practice for Computing the Colors of Objects by Using the CIE System

E313 Practice for Calculating Yellowness and Whiteness Indices from Instrumentally Measured Color Coordinates

E991 Practice for Color Measurement of Fluorescent Specimens Using the One-Monochromator Method

E1164 Practice for Obtaining Spectrometric Data for Object-Color Evaluation

E1331 Test Method for Reflectance Factor and Color by Spectrophotometry Using Hemispherical Geometry

E1348 Test Method for Transmittance and Color by Spectrophotometry Using Hemispherical Geometry

E1349 Test Method for Reflectance Factor and Color by Spectrophotometry Using Bidirectional (45°:0° or 0°:45°) Geometry

E2152 Practice for Computing the Colors of Fluorescent Objects from Bispectral Photometric Data

E2153 Practice for Obtaining Bispectral Photometric Data for Evaluation of Fluorescent Color

3. Terminology

3.1 The definitions in Terminology E284, Practices E991, E2152, and E2153 are applicable to this practice.

4. Significance and Use

4.1 Several standards, including Practices E991, E1164, and Test Methods E1331, E1348 and E1349, require either the presence or absence of fluorescence exhibited by the specimen for correct application. This practice provides spectrophotometric procedures for identifying the presence of fluorescence in materials.

4.2 This practice is applicable to all object-color specimens, whether opaque, translucent, or transparent, meeting the requirements for specimens in the appropriate standards listed in 2.1. Translucent specimens should be measured by reflectance, with a standard non-fluorescent backing material, usually but not necessarily black, placed behind the specimen during measurement.

4.3 This practice requires the use of a spectrophotometer in which the spectral distribution of the illumination on the specimen can be altered by the user in one of several ways. The modification of the illumination can either be by the insertion of optical filters between the illuminating source and the specimen, without interfering with the detection of the radiation from the specimen, or by interchange of the illuminating and detecting systems of the instrument or by scanning of both the illuminating energy and detection output as in the two-monochromator method.

[1] This practice is under the jurisdiction of ASTM Committee E12 on Color and Appearance and is the direct responsibility of Subcommittee E12.05 on Fluorescence.

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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* volume information, refer to the standard's Document Summary page on the ASTM website.

4.4 The confirmation of the presence of fluorescence is made by the comparison of spectral curves, color difference, or single parameter difference such as ΔY between the measurements.

NOTE 2—In editions of E1247 - 92 and earlier, the test of fluorescence was the two sets of spectral transmittances or radiance factor (reflectance factors) differ by 1 % of full scale at the wavelength of greatest difference.

4.5 Either bidirectional or hemispherical instrument geometry may be used in this practice. The instrument must be capable of providing either broadband (white light) irradiation on the specimen or monochromatic irradiation and monochromatic detection.

4.6 This practice describes methods to detect the presence of fluorescence only. It does not address the issue of whether the fluorescence makes a significant or insignificant contribution to the colorimetric properties of the specimen for any given application. The user must determine the practical significance of the effect of fluorescence on the color measurement.

5. Instrumental Requirements

5.1 This practice requires instrumentation meeting the following requirements.

5.1.1 The instrument source shall provide sufficient irradiation energy at the sample port to excite fluorescent emission, if present.

5.1.2 The instrument must provide one of the following illumination/viewing combinations:

5.1.2.1 Monochromatic illumination and monochromatic viewing (that is, a two-monochromator spectrophotometer sometimes called a bispectrometer or spectrofluorimeter).

5.1.2.2 Polychromatic illumination and monochromatic viewing.

5.1.2.3 Reversible illumination/viewing to allow both polychromatic illumination with monochromatic viewing and monochromatic illumination with polychromatic viewing.

5.1.3 The instrument and associated computer software shall allow the standardization/calibration of the instrument using user modified standardization/calibration values, which is a requirement for using any of the filter methods described in this practice.

NOTE 3—Repeatable and accurate application of this practice requires specialized instrumentation. Some commercial one-monochromator spectrometers are limited in their ability to allow for the insertion of optical filters and re-standardization with the filter in place as required in this procedure.

6. Procedures

6.1 There are three general types of procedures to detect the presence of fluorescence instrumentally. Each has its advantages and shortcomings depending on the wavelength and intensity of the fluorescent emission and the instrumentation available to the user.

6.2 *Two-Monochromator Method:* This method requires a colorimetric measuring instrument that is equipped with two separate monochromators: the first, the illumination monochromator, irradiates the specimen with monochromatic light and the second, the viewing monochromator, analyzes the radiation leaving the specimen. A two-dimensional array of bispectral photometric values is obtained by setting the illumination monochromator at a series of fixed wavelengths (μ) in the illumination band of the specimen, and for each μ, using the viewing monochromator to record readings for each wavelength (λ) in the specimen's viewing range. The resulting array, once properly corrected, is known as the Donaldson matrix, and the value of each element (μ,λ) of this array is the Donaldson radiance factor ($D(\mu,\lambda)$). The reflection values are confined to the diagonal of the matrix, and these diagonal values are equal to the spectral reflectance factor of the specimen. Therefore, the presence of fluorescence is demonstrated by non-zero off-diagonal elements. The measurement procedures for this method are given in detail in Practice E2153.

6.3 *Filter Methods:* Filter methods follow the general procedure of making a measurement of spectral radiance factor using a spectrometer with broad band illumination, then adding one or more filters to remove the fluorescence-excitation energy and measuring the spectral radiance factor under the modified illumination. The comparison of the resulting spectral curves shows the presence or absence of fluorescence. If the exclusion of the excitation energy results in a difference in the remaining part of the curve, fluorescence is present and must be considered in the measurement procedures. If no difference is found, then fluorescence is not an issue in the measurement of that specimen.

6.3.1 *UV-Blocking Method*—This procedure is typically used for detecting the presence of optical brighteners, such as in white paper and textiles.

6.3.1.1 Calibrate the instrument as required by the manufacturer. (See Practice E1164 and the appropriate test method for the instrument geometry.)

NOTE 4—Since the measurement will be used to detect fluorescence, it should be considered that fluorescence might be present, therefore the calibration procedure should include adjusting the instrument's illuminator to conform as closely as possible to D65 including the UV region of the spectrum. In some commercial instruments this may be accomplished by calibrating by whiteness index or the UV profile.

6.3.1.2 Measure the specimen, obtaining either a table or a graph of spectral transmittance or reflectance factor versus wavelength.

6.3.1.3 Insert a long-wavelength bandpass filter between the illuminating source and the specimen. Select the cutoff wavelength of the filter according to the color of the specimen using the recommendation in Table 1 as a guide.

(*a*) For spectrophotometers equipped for illumination by means of an integrating sphere, the filter must be placed between the illuminating source and the illumination entrance

TABLE 1 Edge-Position and Emission Wavelengths

Sample Color	Edge-Position Wavelength, nm	Minimum Emission Wavelength, nm
White or blue	440	400
Green	510	480
Yellow	540	480
Orange	620	550
Red	650	560

port of the sphere for reflectance measurement. For transmittance measurement, the filter must be placed between the illuminating source and the specimen.

(*b*) For spectrophotometers equipped for illumination by means of bidirectional geometry, the filter must be placed between the illuminating source and the specimen.

6.3.1.4 Repeat the calibration in accordance with 6.3.1 modifying the calibration values to be 0 below the cutoff of the filter.

6.3.1.5 Repeat the measurement in accordance with 6.3.1.2.

NOTE 5—This method employing only one cut-off filter is most commonly used when measuring white materials where optical brightening is suspected.

6.3.2 *Fluorescence-Weakening Method:* In the fluorescence-weakening method two different bandpass filters are used and three measurements are compared (**1**). One filter is chosen to remove all the fluorescence-exciting wavelengths (fluorescence-killing filter), and the second filter is chosen to remove incident illumination about 20 to 40 nm shorter than the first filter (fluorescence-weakening filter). Use the procedure in 6.3.1.1 and 6.3.1.2 for the measurement without any filter in place. Then use the procedures in 6.3.1.3-6.3.1.5 for the measurements with each of the filters. Refer to the referenced literature for complete details of the application of this method.

6.3.3 *Filter Reduction Method:* Several linear long bandpass filters are placed, one at a time, in the light path between the source and the specimen. Usually 3 to 5 filters are enough to estimate the reflected radiance factor (**2**). The same procedure is used to measure the specimen with each filter in place, following steps 6.3.1.1-6.3.1.5. The difference between the mapped reflected radiance factor and the unfiltered measurement reveals the presence or absence of fluorescence. Refer to the referenced literature for complete details of the application of this method.

6.3.4 *Adjustment Method:* In this method several narrow bandpass filters are placed in the optical path between the source and the specimen one at a time. This produces a series of readings which is used to determine the total radiance factor is a way somewhat analogous to an abridged two-monochromator instrument (**3**), (**4**). Again the difference between the reflectance and the total radiance curves indicates the presence or absence of fluorescence. Follow the procedure in 6.3.1.1-6.3.1.5 for the measurements with each filter. Refer to the referenced literature for complete details of the application of this method.

6.3.5 *Serial Filter Method:* (**5**) This method is a more general case of the filter reduction method and may, with suitable calibration, be equivalent to the two-monochromator method. In the filter reduction method 3 to 5 filters in the region of suspected fluorescence are used. In this method 10 to 12 filters are used to measure the entire visible spectrum. Follow the procedure in 6.3.1.1-6.3.1.5 for measurements with each filter. Then examine the difference between the curves. Refer to the referenced literature for complete details of the application of this method.

6.4 *Two-Mode Method:* The two-mode method also compares the results of two measurements. However in this case, instead of using a filter to exclude the excitation energy, the procedure relies on the fact that the fluorescence will show up as increased values at the emission wavelengths when in the mode involving polychromatic illumination, but not necessarily so when in the mode involving monochromatic illumination. The two spectral curves will always have different shapes when there is fluorescence(**6**),(**7**). Therefore, instruments in which the position of the source and detector can be switched can be used to detect the presence of fluorescence.

6.4.1 Set the instrument for polychromatic illumination and calibrate it, following the instrument manufacturer's instructions. (See Practice E1164 and the appropriate test method for the instrument geometry.)

6.4.2 Measure the specimen, obtaining either a table or a graph of spectral transmittance or reflectance factor versus wavelength.

6.4.3 Set the instrument for monochromatic illumination and calibrate it in a manner similar to that given in 6.3.1.

6.4.4 Measure the specimen in accordance with 6.3.2.

7. Interpretation of Results

7.1 The confirmation of the presence of fluorescence is made by examining the Donaldson matrix or by the comparison of spectral curves at the wavelength of maximum deviation, color difference, or single parameter difference such as ΔY or Whiteness Index (WI) between the measurements. If you have used the two-monochromator method follow step 7.2 or 7.5, or both. If you are using the comparison of spectral curves at the wavelength of maximum deviation follow step 7.3 for the filter methods (6.3) or 7.4 for the two-mode method (6.4). If you are using a color difference calculation or single parameter difference use step 7.5.

7.2 Using the two-monochromator method, examine the Donaldson matrix. Since the reflectance values are confined to the diagonal of the matrix, the presence of fluorescence is indicated by non-zero off-diagonal elements. Alternatively the total radiance curve calculated from the Donaldson matrix (see Practice E2153) can be compared to the reflectance only curve (see 7.3.1 and 7.3.2) or the fluorescence component can be evaluated directly. Finally if desired the colorimetric values, color differences, or whiteness index, or both, can be calculated from this data. (See 7.5.)

7.3 Using the filter methods, examine the tabulated or graphed values of the spectral transmittance or reflectance factor especially in the expected possible emission wavelength region. Table 1 can be used as a guide to the wavelength region to be examined according to the color of the specimen.

7.3.1 For many applications, fluorescence is considered significant if the two sets of spectral transmittances or reflectance factors differ by 1 % at the wavelength of greatest difference based on a scale where a perfect diffusing reflector has a value of 100 %.

7.3.2 The user must determine the level of difference that is significant after considering the purpose of the measurement and the level of precision required.

7.4 Using the two mode method, examine the spectral transmittances or reflectance factors obtained with polychromatic illumination (see 6.4.2 or 6.3.2) and monochromatic

detection to those obtained with monochromatic illumination and polychromatic detection.

7.4.1 For many applications, fluorescence is considered significant if the two sets of spectral transmittances or reflectance factors differ by 1 % at the wavelength of greatest difference based on a scale where a perfect diffusing reflector has a value of 100 %.

7.4.2 The user must determine the level of difference that is significant after considering the purpose of the measurement and the level of precision required.

7.5 Using any of the methods one can calculate the colorimetric values for each set of spectral data. Refer to Practice E308 or Practice E2153 for details. If the change in CIE Y is to be used, compare the Y values. If color difference is to be used, calculate the color difference between the two sets of measurements [the total radiance data and the reflectance (or transmittance) data only] refer to Practice D2244 for details of color difference calculation. If whiteness index is to be considered, then calculate the whiteness index following the procedure in Practice E313 and compare the whiteness index values. The user must determine the level of difference that is significant after considering the purpose of the measurement and the level of precision required.

8. Report

8.1 Identify the specimen and the method used, including the instrumentation and filters, if any.

8.2 State whether or not the specimen was determined as exhibiting fluorescence, and the criteria used.

9. Precision and Bias

9.1 This practice requires no consideration of precision and bias as it reports only the identification of the presence or absence of fluorescence in the specimen. However, the selection of the criterion for fluorescence (see Section 7) is influenced by the precision and bias of the method used. This should be taken into account, and specific statements of precision and bias made if appropriate.

10. Keywords

10.1 fluorescence; light-transmission and reflection; spectrophotometry; transmittance and reflectance

REFERENCES

(1) Allen, E., "Separation of the spectral radiance factor curve of fluorescent substances into reflected and fluoresced components," Applied Optics 12, 1973, pp. 289-293.

(2) Eitel, D., and Ganz, E., "Eine Methode zur Bestimmung von Normfarbvwerten fur fluoreszierende Proben," Textileveredlung, 3, 1968, pp. 389-392.

(3) Blaise, P., "Photometry and colorimetry of retroreflectors and of fluorescent materials," in Proceedings Xth conference of the International Association of Lighthouse Authorities, Tokyo, 1980, IALA, Paris, 1980, paper 6.6.1.

(4) Billmeyer, Jr., F. W., and Chen, Y , "Adjustment Method of Fluorescent Sample Measurement" Color Research and Application 9 1984, pp. 175-178.

(5) Simon, F. T., "Serial filter method for fluorescence evaluation," *Analytica Chimica Acta*, 380, 1999, pp. 417-423.

(6) Grum, F., "Colorimetry of Fluorescent Materials," Color Measurement, Academic Press, New York, Chapter 6, 1980.

(7) Simon, F. T., "The two-mode method for measurement and formulation with fluorescent colorants," *Journal of Color and Appearance*, 1, 1972, pp. 5-11.