



Designation: E275 – 08 (Reapproved 2022)

Standard Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers¹

This standard is issued under the fixed designation E275; 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.

INTRODUCTION

In developing a spectrophotometric method, it is the responsibility of the originator to describe the instrumentation and the performance required to duplicate the precision and accuracy of the method. It is necessary to specify this performance in terms that may be used by others in applications of the method.

The tests and measurements described in this practice are for the purpose of determining the experimental conditions required for a particular analytical method. In using this practice, an analyst has either a particular analysis for which he describes requirements for instrument performance or he expects to test the capability of an instrument to perform a particular analysis. To accomplish either of these objectives, it is necessary that instrument performance be obtained in terms of the factors that control the analysis. Unfortunately, it is true that not all the factors that can affect the results of an analysis are readily measured and easily specified for the various types of spectrophotometric equipment.

Of the many factors that control analytical results, this practice covers verification of the essential parameters of wavelength accuracy, photometric accuracy, stray light, resolution, and characteristics of absorption cells as the parameters of spectrophotometry that are likely to be affected by the analyst in obtaining data. Other important factors, particularly those primarily dependent on instrument design, are also covered in this practice.

1. Scope

1.1 This practice covers the description of requirements of spectrophotometric performance, especially for test methods, and the testing of the adequacy of available equipment for a specific method (for example, qualification for a given application). The tests give a measurement of some of the important parameters controlling results obtained in spectrophotometric methods, but it is specifically not to be concluded that all the factors in instrument performance are measured, or in fact may be required for a given application.

1.1.1 This practice is primarily directed to dispersive spectrophotometers used for transmittance measurements rather than instruments designed for diffuse transmission and diffuse reflection.

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

¹ This practice 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. Referenced Documents

2.1 *ASTM Standards*:²

E131 Terminology Relating to Molecular Spectroscopy

E168 Practices for General Techniques of Infrared Quantitative Analysis

E169 Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis

E387 Test Method for Estimating Stray Radiant Power Ratio of Dispersive Spectrophotometers by the Opaque Filter Method

E958 Practice for Estimation of the Spectral Bandwidth of Ultraviolet-Visible Spectrophotometers

3. Terminology

3.1 *Definitions*:

3.1.1 For definitions of terms used in this practice, refer to Terminology **E131**.

4. Significance and Use

4.1 This practice permits an analyst to compare the general performance of an instrument, as it is being used in a specific spectrophotometric method, with the performance of instruments used in developing the method.

5. Reference to This Practice in Standards

5.1 Reference to this practice in any spectrophotometric test method (preferably in the section on apparatus where the spectrophotometer is described) shall constitute due notification that the adequacy of the spectrophotometer performance is to be evaluated by means of this practice. Performance is considered to be adequate when the instrument can be operated in a manner to give test results equivalent to those obtained on instruments used in establishing the method or in cooperative testing of the method.

5.2 It is recommended that the apparatus be described in terms of the results obtained on application of this practice to instruments used in establishing the method. This description should give a numerical value showing the wavelength accuracy, wavelength repeatability, photometric accuracy, and photometric repeatability found to give acceptable results. A recommended spectral bandwidth maximum should be given along with typical spectra of the components to be determined to indicate the resolution found to be adequate to perform the analysis. If it is considered necessary in a particular analysis, the use of only the linear portion of an analytical curve (absorbance per centimetre versus concentration) may be specified, or if nonlinearity is encountered, the use of special calculation methods may be specified. However, it is not permissible to specify the amount of curvature if a nonlinear working curve is used, because this may vary significantly both with time and the instrument used.

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.

6. Parameters in Spectrophotometry

6.1 Any spectrophotometer may be described as a source of radiant energy, a dispersing optical element, and a detector together with a photometer for measuring relative radiant power. Accurate spectrophotometry involves a large number of interrelated factors that determine the quality of the radiant energy passing through a sample and the sensitivity and linearity with which this radiant energy may be measured. Assuming proper instrumentation and its use, the instrumental factors responsible for inaccuracies in spectrophotometry include resolution, linearity, stray radiant energy, and cell constants. Rigorous measurement of these factors is beyond the scope of this practice. The measurement of stray radiant energy is described in Test Method **E387** and resolution in Practice **E958**.

6.2 Modern spectrophotometers are capable of more accuracy than most analysts obtain. The problem lies in the selection and proper use of instrumentation. In order to ensure proper instrumentation and its use in a specific spectrophotometric method, it is necessary for an analyst to evaluate certain parameters that can control the results obtained. These parameters are wavelength accuracy and precision, photometric accuracy and precision, spectral bandwidth, and absorption-cell constants. Unsatisfactory measurement of any of these parameters may be due to improper instrumentation or to improper use of available instrumentation. It is therefore first necessary to determine that instrument operation is in accordance with the manufacturer's recommendations. Tests shall then be made to determine the performance of an instrument in terms of each of the parameters in **6.1** and **6.2**. Lastly, variations in optical geometry and their effects in realizing satisfactory instrument performance are discussed.

7. Instrument Operation

7.1 In obtaining spectrophotometric data, the analyst must select the proper instrumental operating conditions in order to realize satisfactory instrument performance. Operating conditions for individual instruments are best obtained from the manufacturer's literature because of variations with instrument design. A record should be kept to document the operating conditions selected so that they may be duplicated.

7.2 Because tests for proper instrument operation vary with instrument design, it is necessary to rely on the manufacturer's recommendations. These tests should include documentation of the following factors in instrument operation, or their equivalent:

- 7.2.1 Ambient temperature,
- 7.2.2 Response time,
- 7.2.3 Signal-to-noise ratio,
- 7.2.4 Mechanical repeatability,
- 7.2.5 Scanning parameters for recording instruments, and
- 7.2.6 Instrument stability.

7.3 Each of the factors in instrument operation is important in the measurement of analytical wavelength and photometric data. For example, changes in wavelength precision and accuracy can occur because of variation of ambient temperature of various parts of a monochromator. The correspondence

of the absorbance to wavelength and any internal calculations (or corrections) can affect wavelength measurement for digital instruments. In scanning spectrophotometers, there is always some lag between the recorded reading and the correct reading. It is necessary to select the conditions of operation to make this effect negligible or repeatable. Scanning speeds should be selected to make sure that the detecting system can follow the signal from narrow emission lines or absorption bands. Too rapid scanning may displace the apparent wavelength toward the direction scanned and peak absorbance readings may vary with speed of scanning. A change in instrument response-time may produce apparent wavelength shifts. Mechanical repeatability of the various parts of the monochromator and recording system are important in wavelength measurement. Instructions on obtaining proper mechanical repeatability are usually given in the manufacturer's literature.

7.4 Digital spectrophotometers and diode array spectrophotometers may require a calibration routine to be completed prior to measurement of wavelength or absorbance accuracy. Consult the manufacturer's manual for any such procedures.

WAVELENGTH ACCURACY AND PRECISION

8. Nature of Test

8.1 Most spectrophotometric methods employ pure compounds or known mixtures for the purpose of calibrating instruments photometrically at specified analytical wavelengths. These reference materials may simply be laboratory prepared standards, or certified reference materials (CRMs), where the traceability of the certified wavelength value is to a primary source, either a national reference laboratory or physical standard. The wavelength at which an analysis is made is read from the dial of the monochromator, from the digital readout, from an attached computer, or from a chart in recording instruments. To reproduce measurements properly, it is necessary for the analyst to evaluate and state the uncertainty budget associated with the analytical wavelength chosen.

8.2 The accompanying spectra are given to show the location of selected reference wavelengths which have been found useful. Numerical values are given in wavelength units (nanometres, measured in air). Ref (1)³ tabulates additional reference wavelengths of interest.

9. Definitions

9.1 *wavelength accuracy*—the deviation of the average wavelength reading at an absorption band or emission band from the known wavelength of the band.

9.2 *wavelength precision*—a measure of the ability of a spectrophotometer to return to the same spectral position as measured by an absorption band or emission band of known wavelength when the instrument is reset or read at a given wavelength. The index of precision used in this practice is the standard deviation.

³ The boldface numbers in parentheses refer to a list of references at the end of this standard.

10. Reference Wavelengths in the Ultraviolet Region

10.1 The most convenient spectra for wavelength calibration in the ultraviolet region are the emission spectrum of the low-pressure mercury arc (Fig. 1), the absorption spectra of holmium oxide glass (Fig. 2), holmium oxide solution (Fig. 3), and benzene vapor (Fig. 4). The instrument parameters detailed below these spectra are those used to obtain these reference spectra and may not be appropriate for the system being qualified. Guidance with respect to optimum parameter settings for a given spectrophotometer should be obtained from the instrument vendor or other appropriate reference.

10.2 The mercury emission spectrum is obtained by illuminating the entrance slit of the monochromator with a quartz mercury arc or by a mercury arc that has a transmitting envelope (Note 1). It is not necessary, when using an arc source, that the arc be in focus on the entrance slit of the monochromator. However, it is advantageous to mount the lamp reasonably far from the entrance slit in order to minimize the scatter from the edges of the slit. Reference wavelengths for diode array spectrophotometers can be obtained by placing a low-pressure mercury discharge lamp in the sample compartment. It is not necessary to put the reference source in the lamp compartment for systems with the dispersing element (polychromator) located after the sample compartment.

NOTE 1—Several commercially available mercury arcs are satisfactory, and these may be found already fitted, or available as an accessory from several instrument manufacturers. They may differ, however, in the number of lines observed and in the relative intensities of the lines because of differences in operating conditions. Low-pressure arcs have a high-intensity line at 253.65 nm, and other useful lines as seen in Fig. 1 are satisfactory.

10.3 The absorption spectrum of holmium oxide glass (Fig. 2) is obtained by measuring the transmittance or absorbance of a piece of holmium oxide glass about 2 mm to 4 mm thick.⁴

10.4 The absorption spectrum of holmium oxide solution (Fig. 3) is obtained similarly by measuring an approximately 4 % solution of holmium oxide⁵ in 1.4 M perchloric acid (40 g/L) in a 1 cm cell, with air as reference. For this material, the transmittance minima of 18 absorption bands have been certified by a multi-laboratory inter-comparison, at the highest level, allowing the peak value assignments as an intrinsic wavelength standard (6).

10.5 The absorption spectrum of benzene is obtained by measuring the absorbance of a 1 cm cell filled with vapor (Fig. 4). The sample is prepared by placing 1 or 2 drops of liquid benzene in the cell, pouring out the excess liquid, and stoppering the cell. Some care must be exercised to ensure that the concentration of benzene vapor is low enough to permit resolution of the strongest absorption bands.

NOTE 2—When using complex spectra for wavelength calibration, such as is exhibited by benzene vapor in the ultraviolet, always use the smallest available spectral bandwidth. At bandwidths greater than 0.5 nm, all fine

⁴ Sealed cuvettes of Didymium oxide (1+1 Neodymium and Prasesodymium) and Didymium oxide glass polished filters are available from commercial sources.

⁵ Sealed cuvettes of holmium oxide solution are available from commercial sources and as (the now withdrawn) SRM 2034 from the National Institute of Standards and Technology (5).