Designation: E275 – 08 (Reapproved 2013)

Standard Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers[1]

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

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

[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.

Current edition approved Jan. 1, 2013. Published January 2013. Originally approved in 1965. Last previous edition approved in 2008 as E275 – 08. DOI: 10.1520/E0275-08R13.

[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.

E387 Test Method for Estimating Stray Radiant Power Ratio of Dispersive Spectrophotometers by the Opaque Filter Method
E958 Practice for Measuring Practical 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.

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.

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 (polychomator) 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 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 oxide[5] 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 (**3**).

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 detail, other than the main peaks will be lost (that is, unresolved).

NOTE 3—This test is not recommended for routine use because of the possible health hazards associated with the use of benzene. If the test must be used, it is recommended that the cell be permanently sealed after the concentration of the benzene vapor has been adjusted. Permanently heat-fused cells are commercially available to minimize this risk.

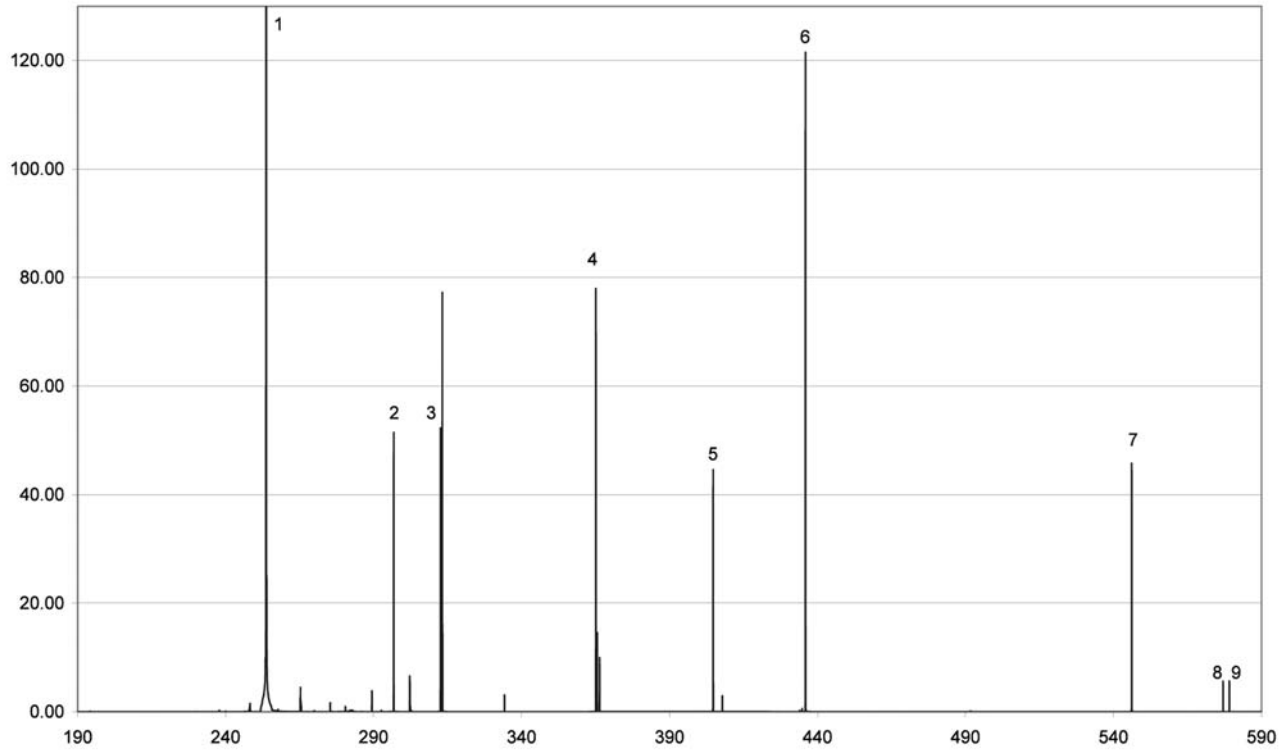
11. Reference Wavelengths in the Visible Region

11.1 In the visible region of the spectrum, calibration wavelengths are obtainable from the mercury emission spectrum (Fig. 1), the absorption spectrum of holmium oxide glass (Fig. 2), the absorption spectrum of holmium oxide in perchloric acid (Fig. 3), or the absorption spectrum of didymium

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

[4] Sealed cuvettes of Didymium oxide (1+1 Neodymium and Praesodymium) 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 (**2**).



Line Number	Wavelength, nm	Line Number	Wavelength, nm	Line Number	Wavelength, nm
1	253.651	4	356.016	7	546.075
2	296.725	5	404.657	8	576.960
3	312.570	6	435.834	9	579.066

Instrument: Cary 5000
Scanning Speed: 1.2 nm/min
Spectral Bandwidth: 0.05 nm
Spectral Data Interval : 0.01 nm

FIG. 1 Mercury Arc Emission Spectrum in the Ultraviolet and Visible Regions Showing Reference Wavelength (4)

solution or glass.[6] If hydrogen or deuterium arc is available, the emission lines 656.3 and 486.1, or 656.1 and 486.0, respectively, can be used.

12. Measurement Procedure

12.1 *Measurement Procedure for Monochromator-Based Spectrophotometers:*

12.1.1 Select two calibration wavelengths, preferably bracketing the analytical wavelength, from those given with the accompanying reference spectra in the region of interest, and observe each wavelength reading ten times (Note 4). Average the observed readings for each wavelength. The wavelength accuracy is the difference between the true wavelength and the average observed reading.

NOTE 4—To check the wavelength accuracy of a nonrecording instrument, balance the instrument at the true value of the absorbance maximum and then adjust the wavelength drive until maximum apparent absorbance has indicated that an accurate setting on the line or band has been achieved. The line or band should always be approached from the same direction.

12.1.2 Calculate the precision of each observed wavelength using the equation:

$$S = \sqrt{\frac{\sum (\lambda_i - \lambda_{aver})^2}{n - 1}} \quad (1)$$

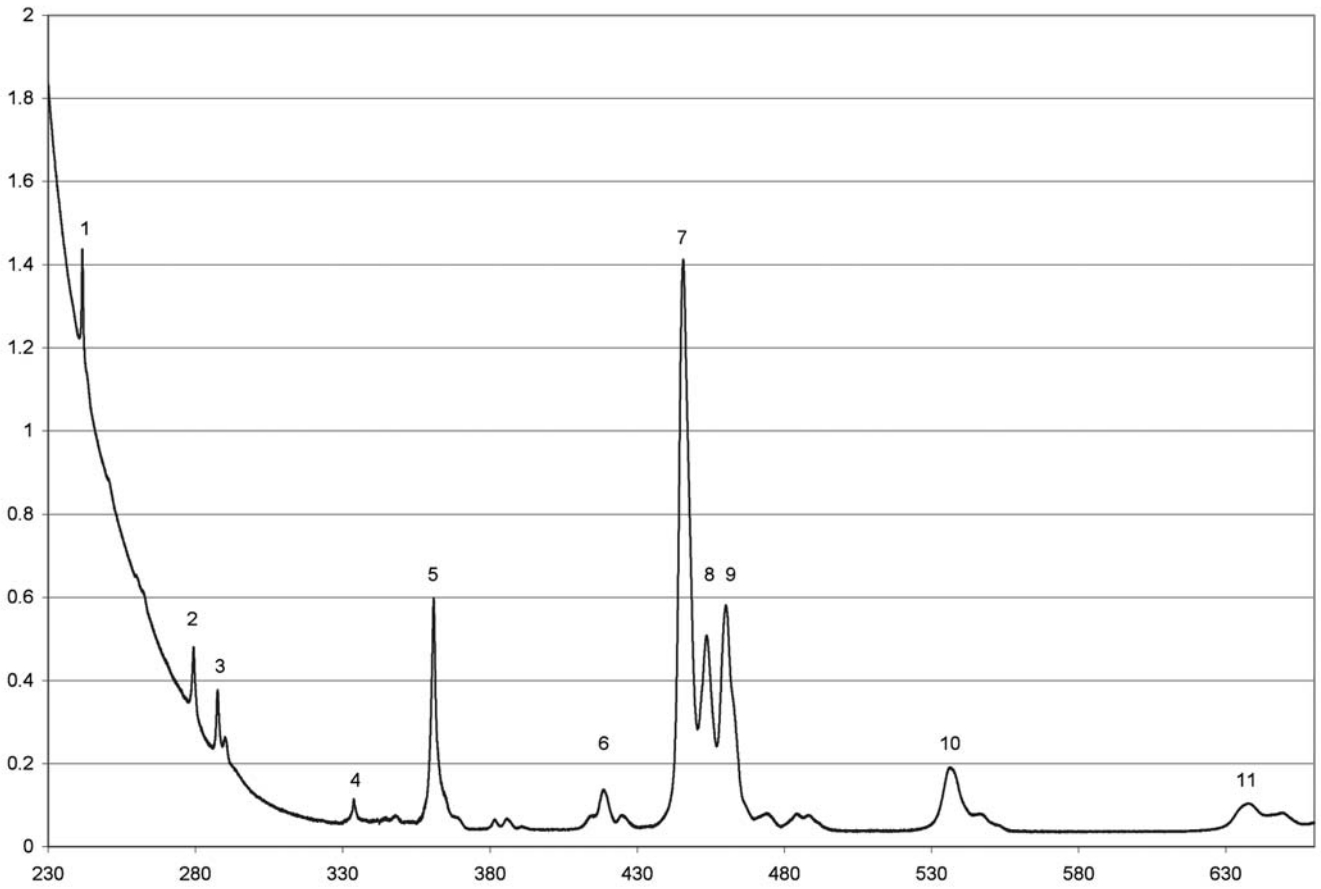
where:

S = standard deviation,
λ_i = individual observed wavelength,
λ_{aver} = averaged observed wavelength, and
n = number of observations (in this case, n = 10).

12.2 *Measurement Procedure for Diode Array Spectrophotometers:*

12.2.1 Acquire ten transmittance spectra of holmium oxide solution or glass or didymium glass. Extract the indicated positions of certified peaks that bracket the analytical wavelength. Average the observed readings for each wavelength. The wavelength bias is the difference between the true wavelength and the average observed reading.

[6] The National Institute of Standards and Technology has supplied didymium glass filters as SRM 2009a. (Detailed information on these filters is presented in Ref (2)).



Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
1	241.64	4	333.86	7	445.67	10	536.27
2	279.38	5	360.92	8	453.62	11	637.69
3	287.55	6	418.66	9	460.21		

Instrument: Cary 5000
Scanning Speed: 0.6 nm/min
Spectral Bandwidth: 0.1 nm
Spectral Data Interval: 0.02 nm

FIG. 2 Spectrum of Holmium Oxide Glass Showing Reference Wavelength (5)

12.2.2 Evaluate precision in the manner of 12.1.2.

12.3 *Specifying Wavelength Accuracy and Wavelength Precision*—Always specify the reference material and the reference wavelength to be used. Results may be expressed conveniently in the following order: reference material (true peak position) and average wavelength plus wavelength standard deviation.

SPECTRAL BANDWIDTH

13. Selection of Spectral Bandwidth

13.1 One of the most important parameters the analyst must select is the spectral bandwidth (if it is adjustable). Many factors in instrument design influence the selection so that it is necessary for an analyst to determine the optimum bandwidth for a particular analysis and instrument.

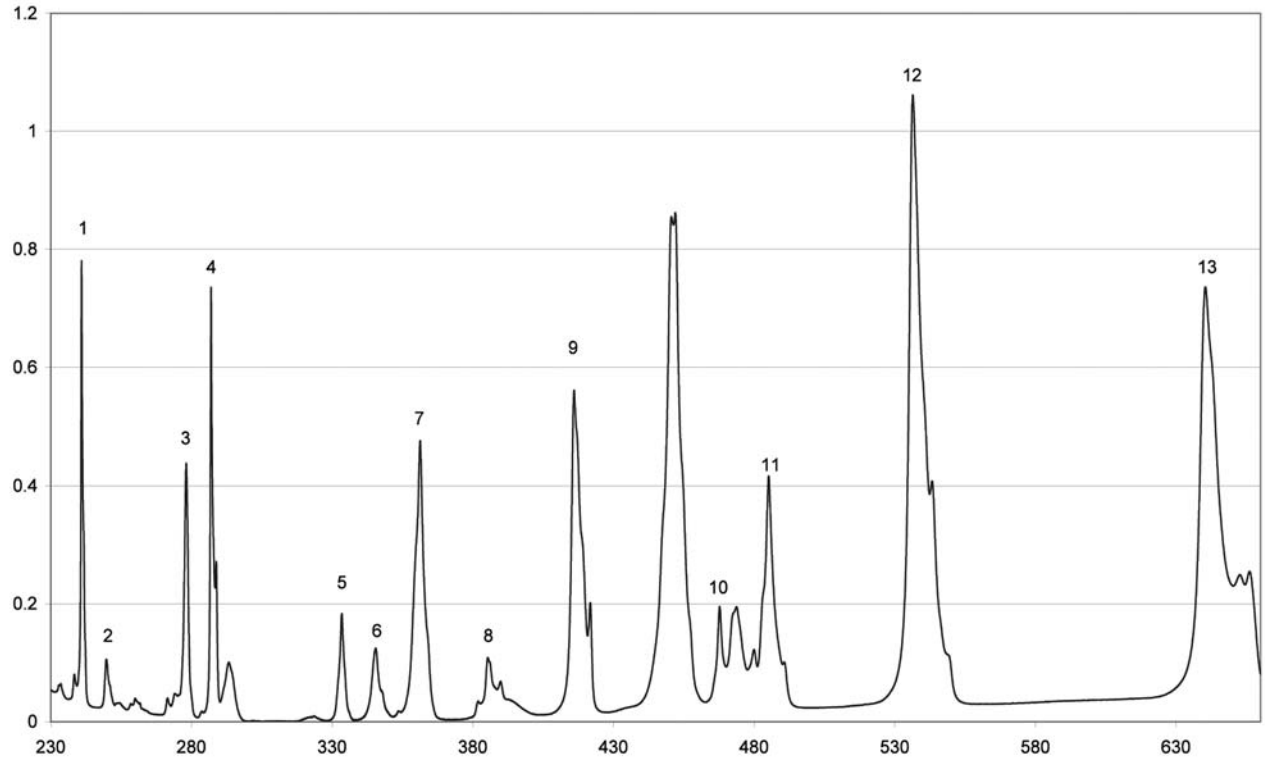
13.2 The optimum spectral bandwidth will be determined by the characteristics of the sample and the dispersion of the instrument used. The narrowest spectral bandwidth should be used that will yield an acceptable signal-to-noise ratio. Where instrument resolution is more than adequate, the signal-to-noise ratio is maximized. In practice, a spectral bandwidth is chosen such that further reduction does not result in a change in absorbance reading.

13.3 The analyst must evaluate the effect that bandwidth has upon resolution as described in Practice E958.

13.4 In each test method involving a spectrophotometric test, typical spectra of the components or a spectrum of a suitable mixture of components should be included to illustrate the resolution found to be adequate to perform the analysis. These spectra should be direct copies of the originals and not redrawn curves.

14. Linearity of Absorbance-Concentration Relationship

14.1 The photometric data an analyst obtains are used to determine concentrations in a spectrophotometric method. It is



Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
1	240.97	5	333.48	9	416.02	13	640.41
2	249.78	6	345.46	10	450.78		
3	278.15	7	361.27	11	452.20		
4	287.03	8	385.36	12	467.42		

Instrument: Cary 5000
Scanning Speed: 0.6 nm/min
Spectral Bandwidth: 0.1 nm
Spectral Data Interval: 0.02 nm

FIG. 3 Spectrum of 4 % Solution of Holmium Oxide in 1.4 *M* Perchloric Acid (1.00-cm Cell) Showing Reference Wavelengths (5)

necessary to establish the relationship between the absorbance and concentration, and to determine the range over which this relationship may be considered linear in calculations.

14.2 In most analyses where the absorption band is completely resolved, there will be a linear relationship between the measured absorbance and the concentration. The range over which this linear relationship applies is determined in part by the performance of the photometric system. In analyses where the absorption band is not completely resolved, or the state of the absorbing component changes with concentration, the relationship between absorbance and concentration may be nonlinear, even on an instrument whose photometric performance would be adequate for a resolved band.

14.3 If nonlinearity is encountered, calculation methods such as those described in Practices E168 must be used. It must be understood, however, that the amount of curvature will depend upon the individual instrument and the particular analysis, and therefore it cannot be specified in a method.

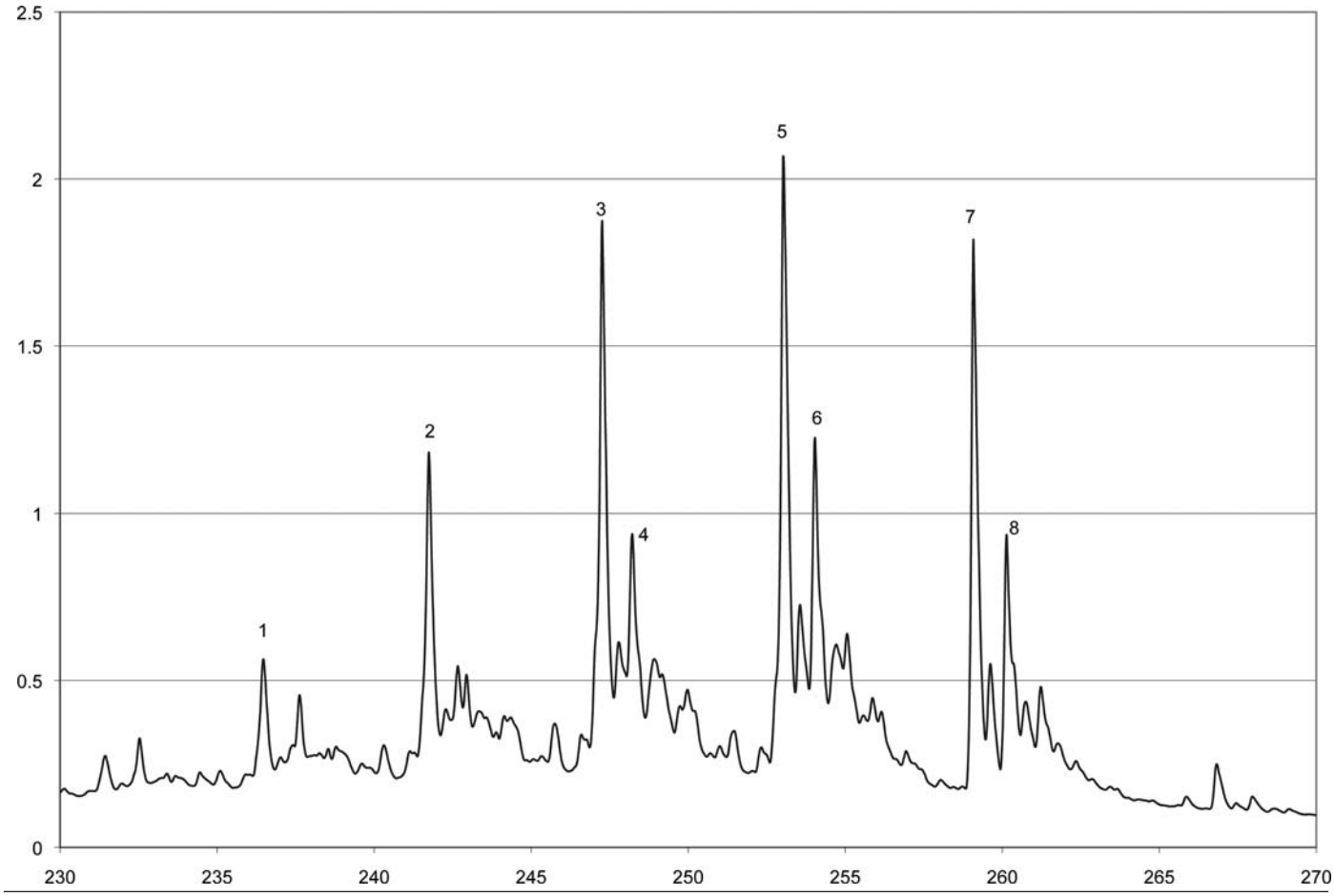
15. Measurement Procedure for Linearity

15.1 Determine the range over which photometry is linear in a particular analysis by preparing an analytical working curve. Descriptions and calculation methods are given in Practices E168 and E169.

15.2 For each component to be determined by a spectrophotometric method, prepare at least three samples containing this component at concentrations that cover the range for which the method is intended. Measure the absorbance at each analytical wavelength for each sample. Prepare an additional set of three samples to obtain two independent sets of data.

15.3 Make a plot of the absorbances as the ordinate and of the concentration as the abscissa. The range of concentrations and absorbances over which a straight line is considered to represent the experimental points is the range over which appropriate linear calculations may be made.

NOTE 5—The required closeness of fit of a straight line to experimental points cannot be specified without reference to a specific analytical



Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
1	236.47	3	247.26	5	253.02	7	259.08
2	241.74	4	248.22	6	254.04	8	260.14

Instrument: Cary 5000
Scanning Speed: 0.6 nm/min
Spectral Bandwidth: 0.1 nm
Spectral Data Interval: 0.02 nm

FIG. 4 Spectrum of Benzene Vapor Showing Selected Reference Wavelengths in the Ultraviolet Region (6)

method. It is necessary to evaluate the data obtained in terms of its effect on the accuracy of the method.

16. Measurement Procedure for Photometric Precision

16.1 In addition to evaluating the range of linearity of the analytical curve, the analyst must determine the precision of the photometric data. Photometric precision represents the capability of the photometer system to reproduce the same value in successive determinations. The index of precision used in this practice is the standard deviation.

16.2 Photometric precision is measured by mounting a suitable known stable reference material, in either cell or filter format in the spectrophotometer, and obtaining ten successive readings of the apparent absorbance or transmittance.

NOTE 6—Screens may only be used singly in the beam. The screen or filter must not be moved during the test and the value obtained must be assumed to be a check only of precision and not of the actual transmittance. Since precision is often a function of the portion of the photometric scale being tested, it is useful to check the performance at a number of points across the scale.

16.3 Tabulate the individual readings of apparent absorbance or transmittance. Average the ten readings. Calculate the standard deviation of ten readings using the following equations:

$$S = \sqrt{\frac{\sum (A_i - A_{aver})^2}{n-1}} \quad \text{or} \quad S = \sqrt{\frac{\sum (T_i - T_{aver})^2}{n-1}} \qquad (2)$$

where:

S = standard deviation,
A_i *and* T_i = individual absorbance or transmittance readings,
A_{aver} *and* T_{aver} = average absorbance or transmittance reading, and
n = number of observations.

16.4 Report the average reading plus or minus the standard deviations for two or more appropriate references. Photometric precision will vary with the transmittance/absorbance being measured and should be measured at least at either end of the measurement range chosen.

17. Photometric Accuracy

17.1 In most analytical applications, photometric accuracy is critical to the robustness of the method, and its ability to be transferred from instrument to instrument.

17.2 Photometric accuracy is determined by using a traceable CRM, where the assigned transmittance values (and associate uncertainty budgets) have been produced by reference to a primary standard, either physical or artifact measured by a national reference laboratory such as the National Institute of Standards and Technology, or other recognized national standards body. Production and value assignment of these materials should be by means of an internationally recognized accreditation standard such as ISO Guide 34 with ISO 17025, or similar

17.3 Photometric accuracy in the visible region can be determined by using neutral density glass filters.

17.4 Photometric accuracy in the ultraviolet region can be determined using acidic potassium dichromate solutions. These can either be of high-purity compounds prepared by the user, potassium dichromate (NIST SRM 935 series), or commercially available sealed-cell format.

17.5 Photometric accuracy in the ultraviolet region can be determined using solutions of high-purity compounds prepared by the user. Molar absorptivities of potassium dichromate (NIST SRM 935 series) in perchloric acid solution at 235, 257, 313, and 350 nm have been published by NIST (**7**). Data for perchloric acid solution of potassium acid phthalate (NIST SRM 84 series) at 262 and 275.5 nm are presented in Ref (**8**). Before using solutions for accuracy checks, one should carefully study the material presented on the effects of concentration, temperature, and pH on the absorptivities.

18. Measurement of Photometric Accuracy

18.1 Select the appropriate CRM and obtain ten successive readings of the apparent absorbance or transmittance at the specified wavelength. Average the ten readings. The photometric accuracy is the difference between the true absorbance or transmittance value and the average observed value.

18.2 Calculate the standard deviation of the observed values using the equations in 16.3.

18.3 Report the photometric accuracy in the following order: reference material, wavelength, true absorbance or transmittance, observed absorbance or transmittance plus or minus the standard deviation.

ABSORPTION CELLS

19. Significance and Use

19.1 The analyst needs to determine that absorption cells serve only as a holder for the sample and do not contribute to the measured absorbance of the sample.

19.2 For precise work, since there are usually small differences among cells, the cells should always be positioned in the same way in the holder and the holder positioned in the same way in the instrument. It should be established that the mechanical repeatability of the sample holder is good enough that it does not introduce a significant error into the analytical procedure. This is best achieved by repeating the photometric precision measurement, but by removing and replacing the cell between each of the ten measurements.

19.3 The most common cause for marked differences between absorption cells is dirty windows. See 20.2 for procedures to test cleanliness. If cells are not properly rinsed, or if the rinsing solution leaves a residue on evaporation, a film may be formed on the window which absorbs part of the radiant energy. When handling cells, care should be taken to avoid touching the windows.

20. Cells for Ultraviolet and Visible Regions

20.1 The most common cell used in this spectral region is the 1-cm liquid cell with glass or silica windows. Other path lengths from 0.001 to 10 cm are commercially available.

NOTE 7—When measurements are made in the ultraviolet, error may derive from fluorescent emission from cell windows and from polarization in the case of crystal-quartz windows.

NOTE 8—The quality of available cells will be reflected in the path length tolerance used in manufacture. Depending on the transmission being measured, this may be significant. For example a 1-cm cell with a ±0.005 cm tolerance will introduce a ±0.005 A error when measuring a solution of 1.0 absorbance.

20.2 *Cleanliness*—To test for cleanliness and gross differences in thickness or parallelism of the optical windows, determine the apparent absorbance of the cell versus air reference as follows:

20.2.1 Fill the cell with distilled water and measure its apparent absorbance against air at 240 nm for quartz cells and at 650 nm for glass cells. With recording instruments, it is desirable to scan over the spectral region of interest. The apparent absorbance should be not greater than 0.093 for 1-cm quartz cells and 0.035 for 1-cm glass cells.

20.2.2 Rotate the cell in its holder (180°) and measure the apparent absorbance again. Rotating the cells should give an absorbance difference not greater than 0.003 A.

NOTE 9—Distilled water and reagent grade methanol are suitable solvents for rinsing cells. If cells become dirty, they can be cleaned by soaking them in water or a mild sulfonic detergent. If residue persists, use of either nitric or hydrochloric acid is permissible up to and including all commercially available acid strengths, providing the appropriate handling precautions are observed. Alkaline solutions, detergents containing "optical bleaches," abrasive powders, fluorides, and materials that might etch the optical windows should be avoided. Do not use ultrasonic baths to clean cells.

20.3 *Cell Correction*—Fill the sample and reference cells with the solvent specified in the test method being used and determine the absorbance of the sample cell at each analytical wavelength. Properly matched cells will have an absorbance difference of less than 0.01. The measured absorbance of the sample cell is the cell correction to be subtracted from absorbance readings of solutions of samples in the same solvent when measured in the same sample cell with the same reference cell.

20.4 *Path Length*—A knowledge of the absolute length of the optical path through the sample in a cell is not essential in analytical procedures as long as the same cells are used in instrument calibration using standard samples and in later

measurements. When determining absorptivities, however, the path length enters into the calculation and must be known. An accurate determination of path length in the 1-cm range is not practical in most laboratories, and common practice is to purchase a cell of known path length.

21. Optical Geometry of the Spectrophotometer

21.1 It is not within the scope of this practice to discuss the fundamental design parameters of any given UV-visible spectrophotometer, but there are a few key parameters that should be reported for any given method, to allow the performance evaluation to be matched to the instrument type.

21.1.1 Beam geometry, that is, single beam where all measurements are performed using the same optical beam, or double beam, where both reference and sample beams are used

21.1.2 Dual/Split beam—where there is a compensating reference beam, but the detector is internal and not readily accessible.

21.1.3 Pre-sample or post-sample dispersion. Check if the monochromator is before or after the sample.

21.1.4 Single or double monochromator—important when establishing the linear range of a system for a given method.

REPORT

22. Report Form

22.1 Report the test results for each analytical wavelength of an analysis using an appropriate report format. An example report is given in Fig. 5.

22.2 Test results are used by originators of methods to describe the spectrophotometric performance used in obtaining cooperative test results. Some judgment must be exercised in making this description reflect the average performance realized by the several laboratories taking part in the cooperative testing. This may be done in the form of a table similar to the report form shown in Fig. 5, or by quoting numerical values showing the range of performance observed if such detailed information is considered advisable. Alternatively, recommendations of a minimum or better performance in the parameters considered to be most important may be made.

22.3 *Example of Apparatus Requirement:*

22.3.1 *Spectrophotometer,* equipped to record automatically absorbance or transmittance of solutions in the spectral region 280 to 320 nm with a spectral bandwidth of 0.5 nm or less. Wavelength measurements shall be repeatable and known to be accurate within ±0.2 nm or less as measured by the mercury emission line at 313.16 nm. In the absorbance range from 0.2 to 1.0, absorbance measurements shall be repeatable within ±1 % or less and in this range absorptivity measurements of the standard sample at the 311-nm absorption peak shall not differ by more than 2 % from their average value.

NOTE 10—An instrument is considered suitable when it can be operated in a manner to give test results which match the user defined Apparatus Requirement specification.

22.3.2 *Quartz Cells,* two, having a sample path length known to be in the range from 1.000 ± 0.005 cm.

23. Keywords

23.1 molecular spectroscopy; spectroscopy; ultraviolet spectrophotometer; visible spectrophotometer

Spectrophotometer Performance Report

Instrument Description

Manufacturer: Serial Number:

Model:

Beam Geometry: (Single/Dual/Double) Monochromator (Single/Double) (Pre/Post Sample)

Instrument Performance

Wavelength accuracy Mean value +/- Standard Deviation	*Photometric accuracy* Mean value +/- Standard Deviation
Wavelength precision Mean value +/- Standard Deviation	*Photometric precision* Mean value +/- Standard Deviation
Reference Material (1) (2) (3) Certified values	Reference Material (1) (2) (3) Certified values
Stray Light: Reference Material Measured value	*Resolution:* Reference Material Measured value

Analytical Parameters

Sample:

Wavelength: Allowed wavelength uncertainty:

Linear range (Absorbance): Spectral Bandwidth:

Linear range (Concentration): Cell pathlength:

Tested by: **Location:**

Date:

Comments:

FIG. 5 Report Form

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