Designation: E 275 – 01

Standard Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers[1]

This standard is issued under the fixed designation E 275; 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.

This standard has been approved for use by agencies of the Department of Defense.

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 selection of the setting of analytical wavelength, selection of slit width, photometric measurements, 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 not covered in this practice.

1. Scope

1.1 This practice covers the description of requirements of spectrophotometric performance especially for ASTM methods, and the testing of the adequacy of available equipment for a specific method. 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.

1.1.1 This practice is not to be used (*1*) as a rigorous test of performance of instrumentation, or (*2*) to intercompare the quantitative performance of instruments of different design.

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

2. Referenced Documents

2.1 *ASTM Standards:*

E 131 Terminology Relating to Molecular Spectroscopy[2]

E 168 Practices for General Techniques of Infrared Quantitative Analysis[2]

E 169 Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis[2]

E 387 Test Method for Estimating Stray Radiant Power Ratio of Spectrophotometers by the Opaque Filter Method[2]

E 958 Practice for Measuring Practical Spectral Bandwidth of Ultraviolet—Visable Spectrophotometers[2]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this practice, refer to Terminology E 131.

4. Significance and Use

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

[1] This practice is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.01 on Ultraviolet and Visible Spectroscopy.

Current edition approved Feb. 10, 2001. Published April 2001. Originally published as E 275 – 65 T. Last previous edition E 275 – 93.

[2] *Annual Book of ASTM Standards,* Vol 03.06.

5. Reference to This Practice in Standards

5.1 Reference to this practice in any ASTM spectrophotometric 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, and photometric repeatability found to give acceptable results. A recommended spectral slit width 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.

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 Method E 387.

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, spectral slit width, photometry, 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.

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 will displace the apparent wavelength toward the direction scanned and peak absorbance readings will 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. 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 state the wavelength limits within which the analytical wavelength is known.

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 or micrometres, measured in air). Reference **(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).

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. Displacement of the source will not shift the apparent wavelength as long as the slit widths used are small, that is, less than 0.1 mm. 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. 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.

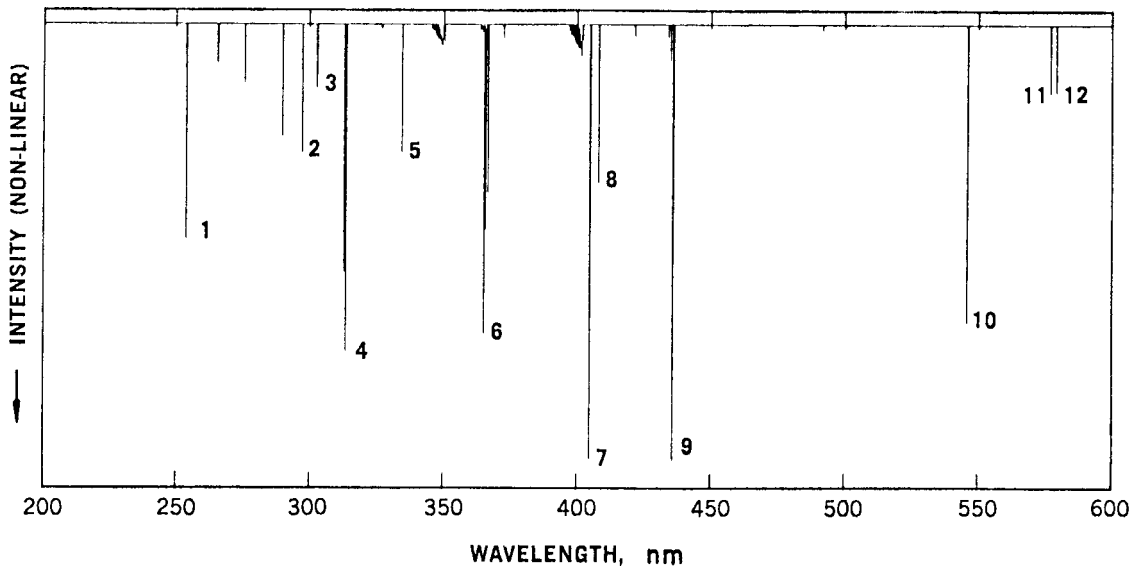
10.3 The absorption spectrum of holmium oxide glass (Fig. 2) is obtained by measuring the transmittance or absorbance of a piece of holmium oxide glass about 2 to 4 mm thick.[4] The absorption spectrum of holmium oxide solution (Fig. 3) is obtained similarly by measuring an approximately 4 % solution of holmium oxide[5] in 1.4 *M* perchloric acid (40 g/L) with air as reference.

10.4 The absorption spectrum of benzene is obtained by measuring the absorbance of a 1-cm cell filled with vapor (Fig. 6). 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

[3] The boldface numbers in parentheses refer to the list of references appended to this practice.

[4] Holmium oxide glass is available commercially as a polished filter.

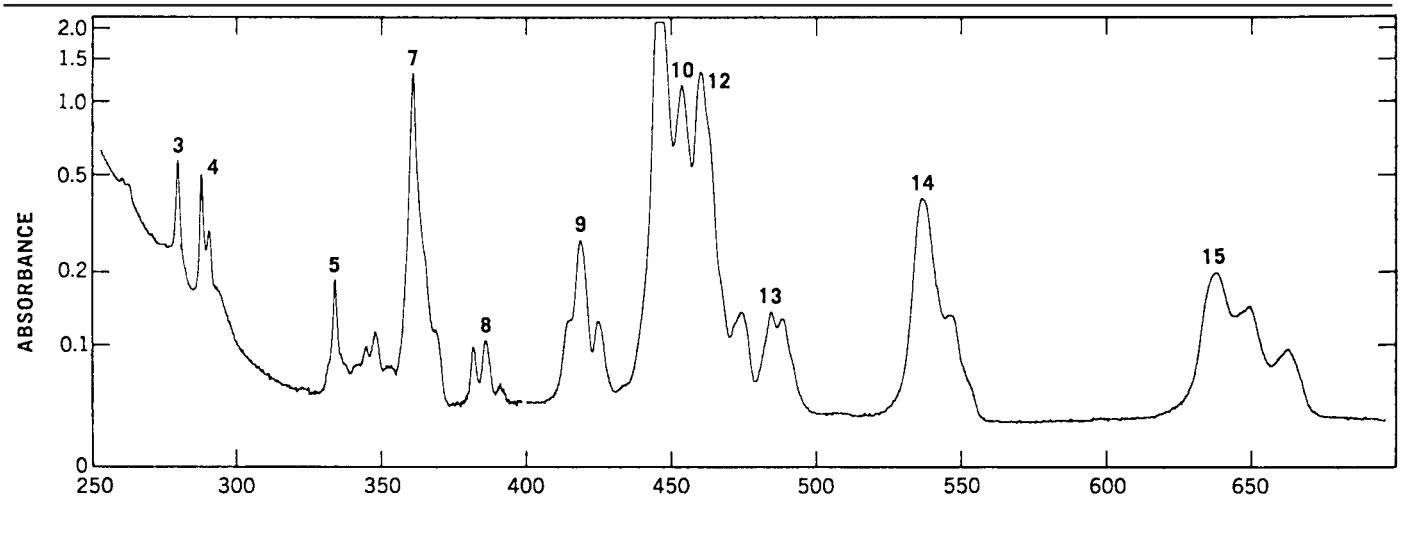
[5] Sealed cuvettes of holmium oxide solution are available from commercial sources and from the National Institute of Standards and Technology (as SRM 2034 (**2**)).



Line Number	Wavelength, nm	Line Number	Wavelength, nm	Line Number	Wavelength, nm	Line Number	Wavelength, nm
1	253.65	4	313.16	7	404.66	10	546.07
2	296.73	5	334.15	8	407.78	11	576.96
3	302.15	6	365.01	9	435.84	12	579.07

Instrument: Cary Model 14
Scanning Speed: 2.5 A/s
Slit Width: 0.03 mm
Spectral Slit Width: 0.10 to 0.15 nm

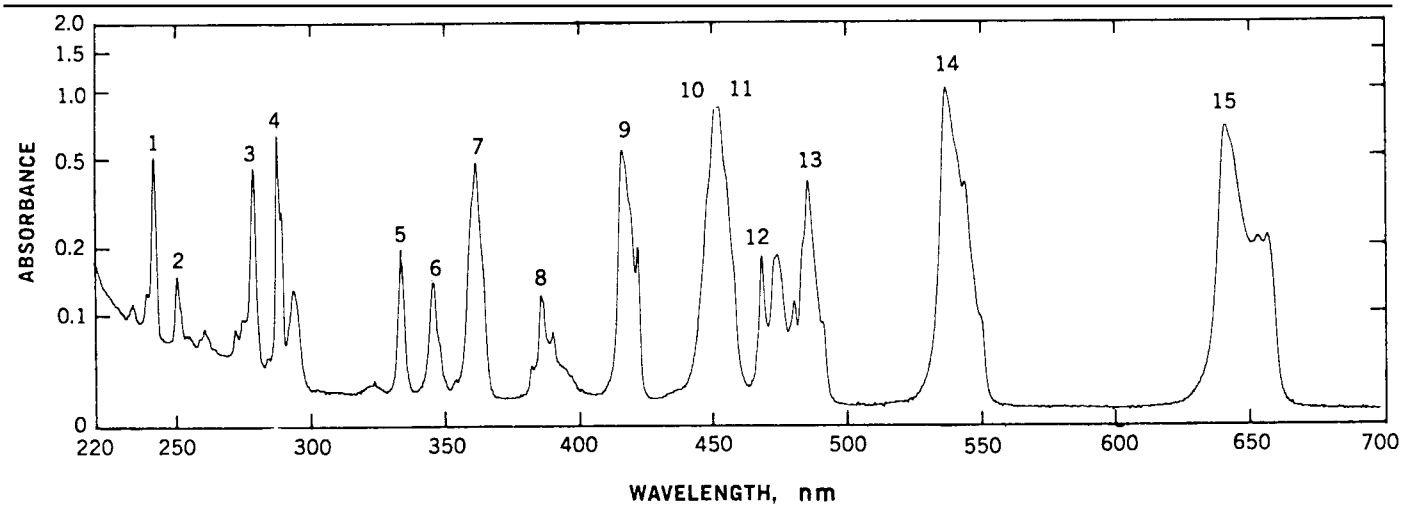
FIG. 1 Mercury Arc Emission Spectrum in the Ultraviolet and Visible Regions Showing Reference Wavelength (4)



Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
3	279.4	7	360.9	10	453.2	14	536.2
4	287.5	8	385.9	12	460.0	15	637.5
5	333.7	9	418.7	13	484.5		

Instrument: Cary Model 14
Scanning Speed: 10A/s
Slit Width: 0.025 to 0.105 mm
Spectral Slit Width: 0.10 to 0.40 nm
Sample Thickness: 2.6 mm

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



Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
1	241.1	5	333.4	9	416.3	13	485.8
2	249.7	6	345.5	10	450.8	14	536.4
3	278.7	7	361.5	11	452.3	15	641.1
4	287.1	8	385.4	12	467.6		

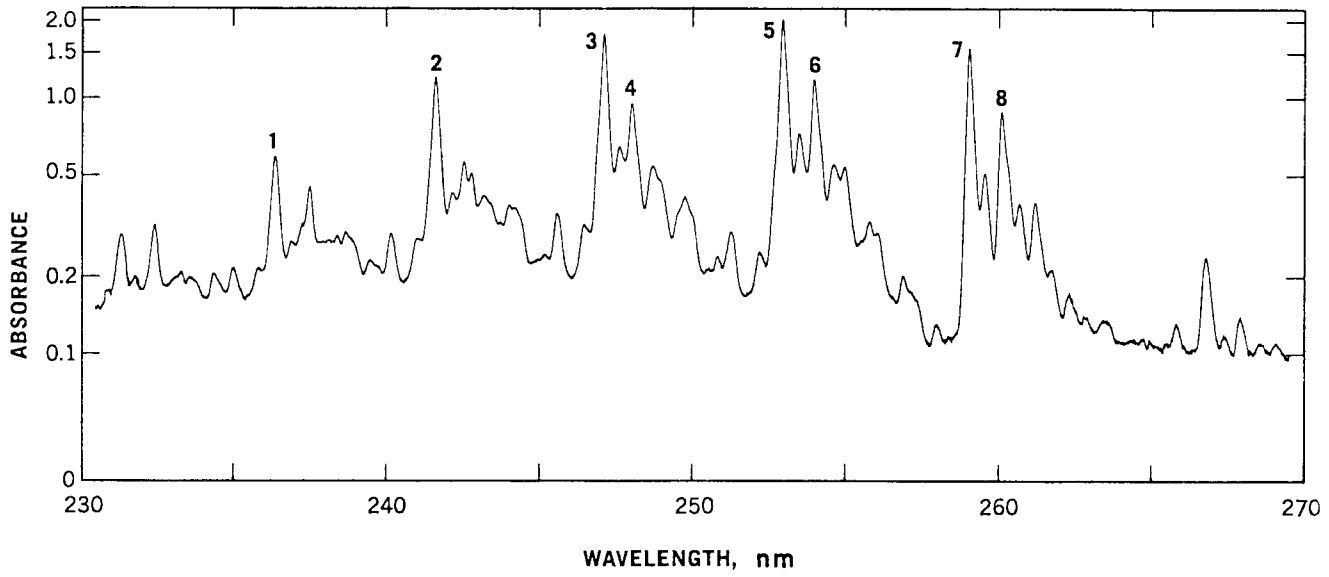
Instrument: Cary Model 14
Scanning Speed: 10A/s
Slit Width (Visible): 0.02 to 0.10 mm
(Ultraviolet): 0.09 to 0.40 mm
Spectral Slit Width (Visible): 0.1 to 0.4 nm
(Ultraviolet): 0.3 to 0.7 nm
Reference: 1.4 *M* Perchloric acid in 1-cm cell

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

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, the approximate conditions of resolution used in obtaining the reference spectra must be achieved in order to depend upon the wavelength values.

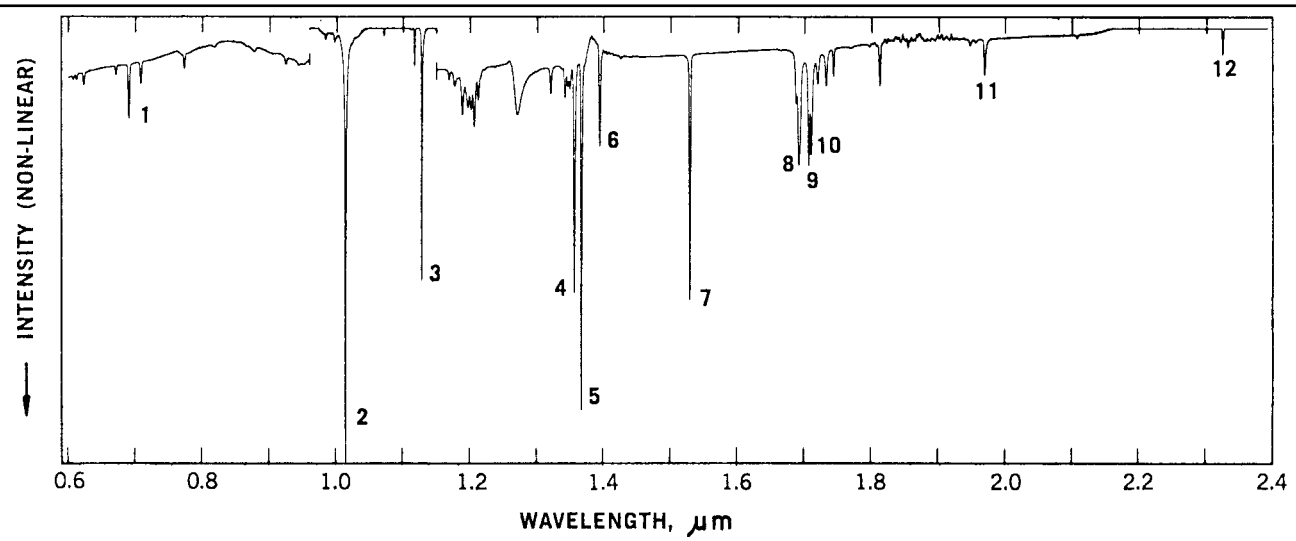
NOTE 3—This test is not recommended for routine use because of the possible health hazards associated with the use of benzene. If the test must be used, it is recommended that the cell be permanently sealed after the concentration of the benzene vapor has been adjusted.



Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
1	236.35	3	247.10	5	252.86	7	258.90
2	241.59	4	248.08	6	253.90	8	259.98

Instrument: Cary Model 14
Scanning Speed: 0.5 A/s
Slit Width: 0.07 mm
Spectral Slit Width: 0.17 nm
Cell Length: 1 cm

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



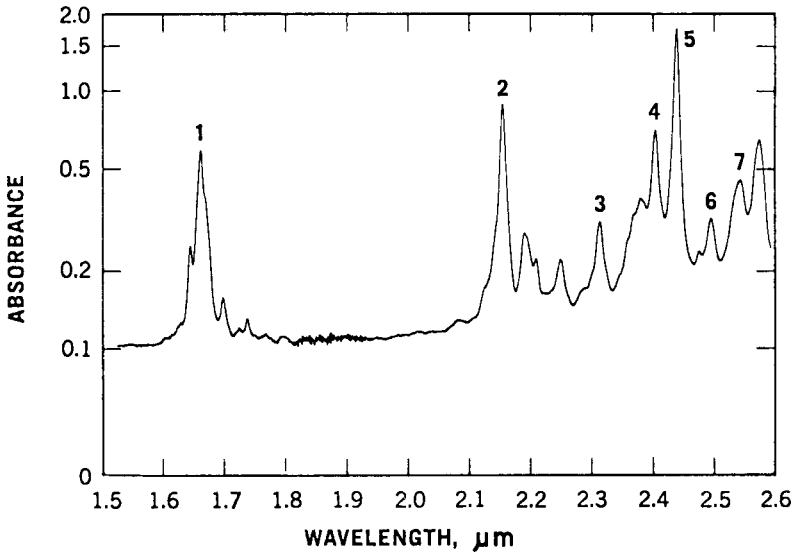
Line	Wavelength (λ), μm	Line	Wavelength (λ), μm	Line	Wavelength (λ), μm	Line	Wavelength (λ),μm
1	0.6907	4	1.3570	7	1.5295	10	1.7110
2	1.0140	5	1.3673	8	1.6921	11	1.9701
3	1.1287	6	1.3950	9	1.7073	12	2.3253

Instrument: Cary Model 14
Scanning Speed: 10 A/s
Slit Width: 0.2 mm for Lines 2 and 3;
0.5 mm for other lines
Spectral Slit Width: 0.001 μm for Lines 2 and 3;
0.002 μm for other lines

FIG. 5 Emission Spectrum of High-Pressure Mercury Arc Showing Reference Wavelengths (4)

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



Band Number	Wavelength, µm	Band Number	Wavelength, µm	Band Number	Wavelength, µm	Band Number	Wavelength,µ m
1	1.6606	3	2.3126	5	2.4374	7	2.543
2	2.1526	4	2.4030	6	2.494		

Instrument: Cary Model 14
Scanning Speed: 25 A/s
Slit Width: 0.21 to 1.80 mm
Spectral Slit Width: 0.001 to 0.005 µm
Cell Thickness: 0.1 cm

FIG. 6 Spectrum of 1, 2, 4-Trichlorobenzene Showing Reference Wavelengths in the Near-Infrared Region (11)

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. Reference Wavelengths in the Near-Infrared Region

12.1 The near-infrared spectral region may be calibrated using a high-pressure mercury arc (Fig. 5), the absorption spectrum of 1,2,4-trichlorobenzene (Fig. 6), or the absorption spectrum of SRM 2035 (rare-earth oxides in glass).[6]

12.2 The high-pressure mercury arc spectrum **(4,5)** is obtained in the same manner as described for the low-pressure mercury arc (10.2). The absorption spectrum of 1,2,4-trichlorobenzene in the near-infrared is obtained in a 0.1-cm cell filled with liquid sample. The absorption spectrum of SRM 2035 is referred to air.

13. Measurement Procedure

13.1 *Measurement Procedure for Monochromator-Based Spectrophotometers*:

13.1.1 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.

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

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

where:
S = standard deviation,
λ_i = individual observed wavelength,
$\lambda_{a\ ver}$ = averaged observed wavelength, and
n = number of observations (in this case, n = 10).

13.2 *Measurement Procedure for Diode Array Spectrophotometers*:

13.2.1 Acquire ten transmittance spectra of holmium oxide glass or solution, didymium glass, or the mixed rare-earth oxide NIR 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.

13.2.2 Evaluate precision in the manner of 13.1.2.

13.3 *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

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

peak position) and average wavelength plus wavelength standard deviation.

SPECTRAL SLIT WIDTH

14. Selection of Slit Width

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

14.2 The optimum slit width will be determined by the spectral characteristics of the sample and the dispersion of the instrument used (ignoring variation among detectors). The narrowest slit width 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.

14.3 The analyst must evaluate the effect that slit width has upon resolution as described in Practice E 958. Alternatively, spectral slit width provides an expedient means of expressing theoretical resolution.

15. Calculation of Spectral Slit Width

15.1 At each analytical wavelength, record the slit width used in millimetres and obtain from the manufacturer's literature the linear dispersion in millimetre per unit wavelength or its reciprocal—wavelength units per millimetre (Note 5). Calculate the spectral slit width by multiplying the slit width in millimetres by the reciprocal linear dispersion in wavelength units per millimetre (Note 6).

NOTE 5—For most instruments the linear dispersion is a function of the wavelength and is available in the manufacturer's instruction manual. If the slit width is less than about 0.1 mm, the value obtained for the spectral slit width may not be significant unless some account is taken of factors limiting resolution such as slit curvature and diffraction effects.

NOTE 6—It is good practice to check that the slit width readings are not subject to a "zero" correction. The manufacturer's literature usually describes such a test which demonstrates that slit widths (greater than 0.1 mm) are linearly proportional to the square root of radiant energy and that a plot of the data produces a line that can be extrapolated to pass through zero slit width.

15.2 In each ASTM 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.

PHOTOMETRY

16. Linearity of Absorbance-Concentration Relationship

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

16.2 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.

16.3 If nonlinearity is encountered, calculation methods such as those described in Practices E 168 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.

17. Measurement Procedure for Linearity

17.1 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 E 168 and E 169.

17.2 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.

17.3 Make a plot of the absorbances as the ordinate and of the concentration as the abscissa. (See, for example, Fig. 2 of Practices E 168.) 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 7—The required closeness of fit of a straight line to experimental points cannot be specified without reference to a specific analytical method. It is necessary to evaluate the data obtained in terms of its effect on the accuracy of the method.

18. Measurement Procedure for Photometric Precision

18.1 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.

18.2 Photometric precision is measured by mounting a suitable perforated metallic screen or glass filter (see 19.3), in the spectrophotometer and obtaining ten successive readings of the apparent absorbance or transmittance.

NOTE 8—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.

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

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

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.

18.4 Report the average reading plus or minus the standard deviations for one or more screens or glass filters. Unless otherwise specified, report the readings for a screen or filter, which has a nominal transmittance of 0.4.

19. Photometric Accuracy

19.1 In many analytical applications, photometric accuracy is not critical as long as the photometric readings are precise and yield a linear absorbance-concentration relation over a reasonable range. In other applications, notably those requiring the comparison of absorptivities measured on different instruments, the photometric accuracy of an instrument becomes an important property.

19.2 Photometric accuracy is determined by using one or more samples whose transmittance has been accurately measured by a standardizing laboratory, such as the National Institute of Standards and Technology.

19.3 Photometric accuracy in the visible region can be determined by using NIST Standard Reference Material 930, which is a set of three neutral density glass filters having nominal transmittances of 10, 20, and 30 %. Alternatively, one can use NBS Standard Reference Material 931, which is a set of liquid filters prepared by dissolving high-purity cobalt and nickel in a mixture of nitric and perchloric acids **(7)**.

19.4 Photometric accuracy in the ultraviolet and visible regions can be determined using NIST Standard Reference Material 2031, which is a set of metal-on-quartz filters having nominal transmittances of 90, 30, and 10 % **(8)**.

19.5 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 the National Institute of Standards and Technology **(9)**. Data for perchloric acid solution of potassium acid phthalate (NIST SRM 84 series) at 262 and 275.5 nm are presented in (**10**). Before using solutions for accuracy checks, one should carefully study the material presented on the effects of concentration, temperature, and pH on the absorptivities.

20. Measurement of Photometric Accuracy

20.1 Select the appropriate Standard Reference Material 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.

20.2 Calculate the standard deviation of the observed values using the equations in 18.3.

20.3 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

21. Significance and Use

21.1 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.

21.2 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.

21.3 The most common cause for marked differences between absorption cells is dirty windows. See 22.2 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.

22. Cells for Ultraviolet, Visible, and Near-Infrared Regions

22.1 The most common cell used in this spectral region is the 1-cm liquid cell with glass or silica windows. Other path lengths are equally useful.

NOTE 9—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. Cells denoted as "UV quartz" may have significant absorption in the near infrared.

22.2 *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:

22.2.1 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.

22.2.2 Rotate the cell in its holder (180°) and measure the apparent absorbance again. Rotating the cells should give an absorbance difference not greater than 0.005.

NOTE 10—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, a mixture consisting of 1 volume of concentrated hydrochloric acid (HCl, sp gr 1.19), 3 volumes of water, and 4 volumes of methanol is recommended. (**Warning**—This mixture should be prepared and used only under the hood.) For fused-silica cells, soaking in chromic acid cleaning solution is used by many but care must be taken to rinse the cells with water immediately after removing from the cleaning solution. Alkaline solutions, detergents containing" optical bleaches," abrasive powders, fluorides, and materials that might etch the optical windows should be avoided.

22.3 *Cell Correction*—Fill the sample and reference cells with the solvent specified in the ASTM method being used and

Analytical wavelength _____

_____ ( ), _____ ± _____
Reference material | True peak position | Average wavelength | Standard deviation

_____ ( ), _____ ± _____
Reference material | True peak position | Average wavelength | Standard deviation

Slit width at analytical wavelength _____ mm

Reciprocal linear dispersion _____
wavelength units per mm

Sample _____

Linear range of analytical curve _____ _____
Absorbance units | Concentration units

Photometric precision _____ ± _____
Average value | Standard deviation

Photometric accuracy _____ ( ), _____ ± _____
Reference material | True value | Average value | Standard deviation

Absorption-cell pathlength _____ mm

Instrument description _____ _____ _____
Manufacturer | Model No. | Serial No.

Test by _____ Affiliation _____ Date _____

Comment _____

FIG. 7 Report Form

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.

22.4 *Pathlength*—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 pathlength enters into the calculation and must be known. An accurate determination of pathlength in the 1-cm range is not practical in most laboratories, and common practice is to purchase a cell of known path length.

REPORT

23. Report Form

23.1 Report the test results for each analytical wavelength of an analysis in accordance with Fig. 7.

23.2 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. 7, 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. For example, 23.3 gives a description of instrumentation for a hypothetical ultraviolet spectroscopic method that measures naphthalene absorbance at 311 nm and in which linearity of the analytical curve is considered important. The numerical values given are not significant since they do not apply to an actual analytical method.

23.3 *Example of Apparatus Requirement*:

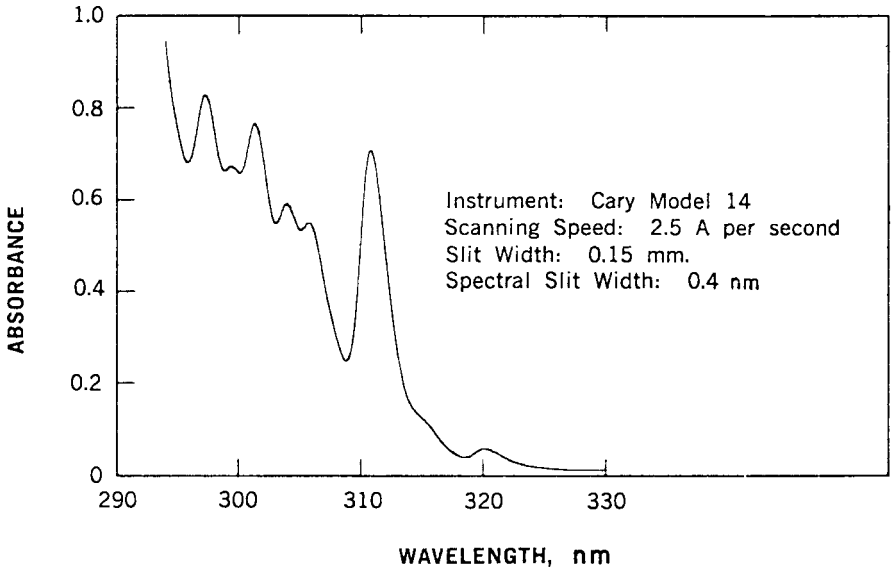
23.3.1 *Spectrophotometer*, equipped to record automatically absorbance or transmittance of solutions in the spectral region 280 to 320 nm with a spectral slit width of 0.5 nm or less. Wavelength measurements shall be repeatable and known to be accurate within ±0.2 nm or less as measured by the mercury 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 11—An instrument is considered suitable when it can be operated in a manner to give test results equivalent to those illustrated in 23.3.1. The attached spectrum of naphthalene in *iso*octane shown in Fig. 8 illustrates resolution found to be adequate to perform this analysis.

23.3.2 *Silica Cells*, two, having a sample pathlength known to be in the range from 1.000 ± 0.005 cm.

24. Keywords

24.1 molecular spectroscopy; near-infrared spectrophotometers; spectroscopy; ultraviolet spectrophotometer; visible spectrophotometer



Concentration: 0.35 g/L Cell Thickness: 1 cm

FIG. 8 Ultraviolet Spectrum of Naphthalene in 2,2,4-Trimethylpentane Solution

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