



# Standard Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers<sup>1</sup>

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 ( $\epsilon$ ) 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.

## 2. Referenced Documents

### 2.1 ASTM Standards:

- E 131 Terminology Relating to Molecular Spectroscopy<sup>2</sup>
- E 168 Practices for General Techniques of Infrared Quantitative Analysis<sup>2</sup>

<sup>1</sup> This practice is under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.01 on Ultraviolet and Visible Spectroscopy.

Current edition approved Dec. 15, 1993. Published February 1994. Originally published as E 275 – 65 T. Last previous edition E 275 – 83 (1989).

<sup>2</sup> Annual Book of ASTM Standards, Vol 03.06.

E 169 Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis<sup>2</sup>

E 387 Test Method for Estimating Stray Radiant Power Ratio of Spectrophotometers by the Opaque Filter Method<sup>2</sup>

## 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.

## 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 are 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 a measurement 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 speed for recording instruments, and
- 7.2.6 Optical stability (for diode array).

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, particularly a crystal prism. The correspondence of the absorbance to wavelength and any internal calculations (or corrections) can affect wavelength measurement for digital instruments. In recording 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 and positioning of chart paper are important in wavelength measurement. Successive batches of chart paper should be checked for uniformity of the chart spectrum length, particularly if the paper has been subjected to pronounced humidity changes. 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)**<sup>3</sup> tabulates additional reference wavelengths of interest.

<sup>3</sup> The boldface numbers in parentheses refer to the list of references appended to this practice.

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 dispers-

ing element (polychromator) 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 such as the Beckman arc<sup>4</sup> and the “Pen Ray” quartz lamp or common germicidal lamps with transmitting envelopes have a high-intensity line at 253.65 nm, and other useful lines as seen in Fig. 1 are satisfactory.

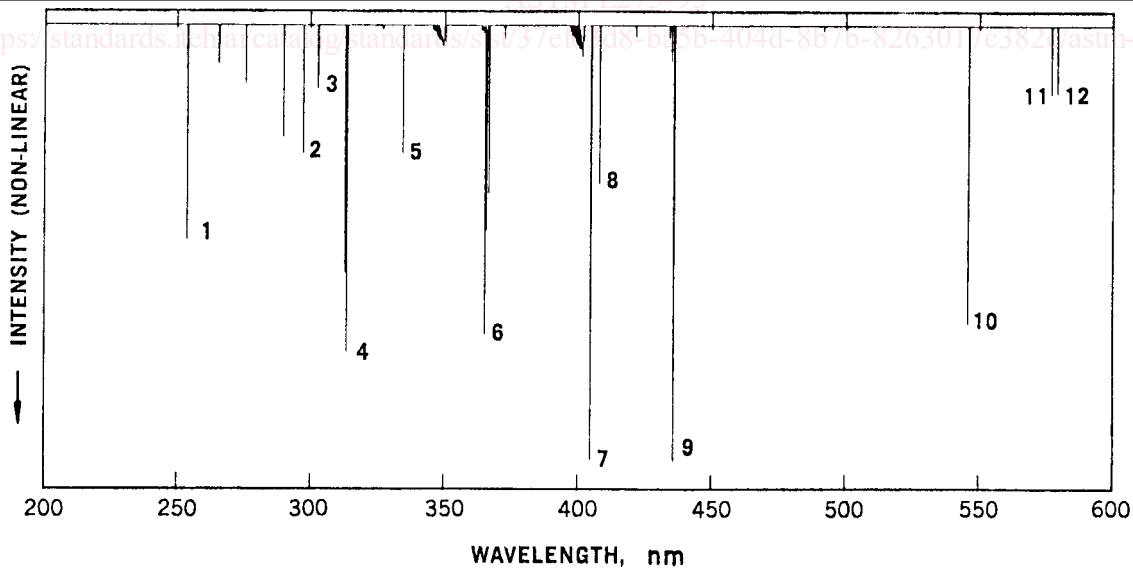
10.3 The absorption spectrum of holmium oxide glass (Fig. 2) is obtained by measuring the transmittance or absorbance of a piece of holmium oxide glass about 2 to 4 mm thick.<sup>5</sup> The absorption spectrum of holmium oxide solution (Fig. 3) is obtained similarly by measuring an approximately 4 % solution of holmium oxide<sup>6</sup> 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. 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

<sup>4</sup> The Beckman mercury arc is manufactured by Beckman Instruments, Inc., Fullerton, Calif., and is most useful on the Beckman DU Spectrophotometer. The “Pen Ray” quartz lamp is manufactured by Ultraviolet Products, Inc., San Gabriel, Calif. Its small size makes it convenient to use. Both are available from equipment distributors.

<sup>5</sup> Holmium oxide glass is available as a polished filter, 2-mm thick, in 2-in. (51-mm) squares designated as Corning Color Filter CS3-142, Glass No. 3131, from F.J. Gray & Co., 139 Queens Blvd., Jamaica, NY 11435, and other industrial materials distributors.

<sup>6</sup> Holmium oxide may be obtained from Lindsay Chemical Division, American Potash and Chemical Corp., West Chicago, IL.

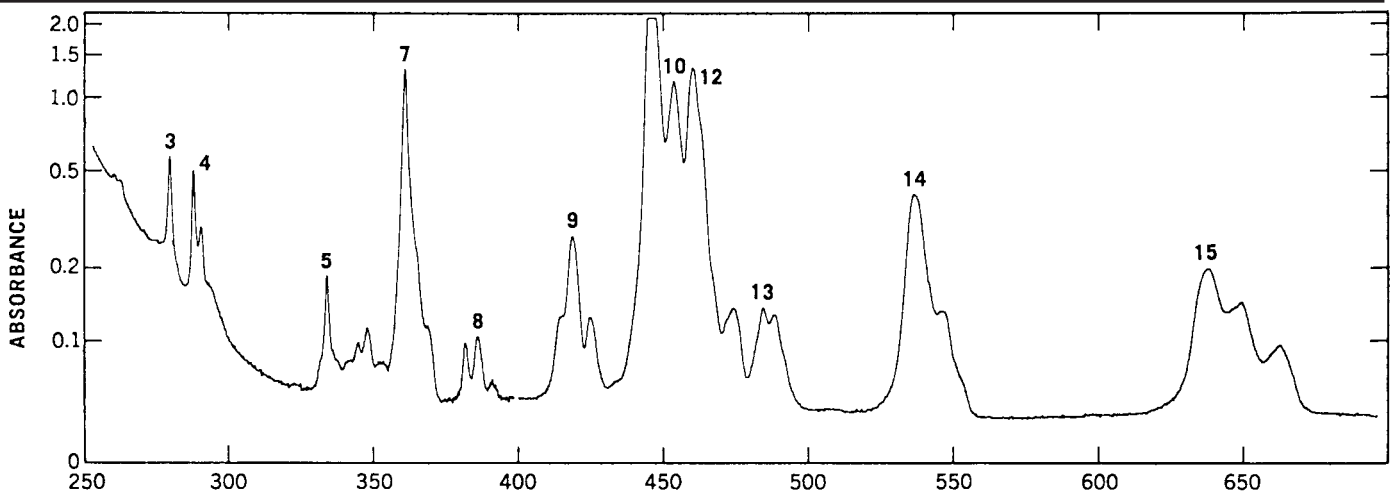


Line Number	Wavelength, nm	Line Number	Wavelength, nm	Line Number	Wavelength, nm	Line Number	Wavelength, nm
1	253.65	4	313.16	7	404.66	10	546.07
2	296.73	5	334.15	8	407.78	11	576.96
3	302.15	6	365.01	9	435.84	12	579.07

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<sup>9</sup>

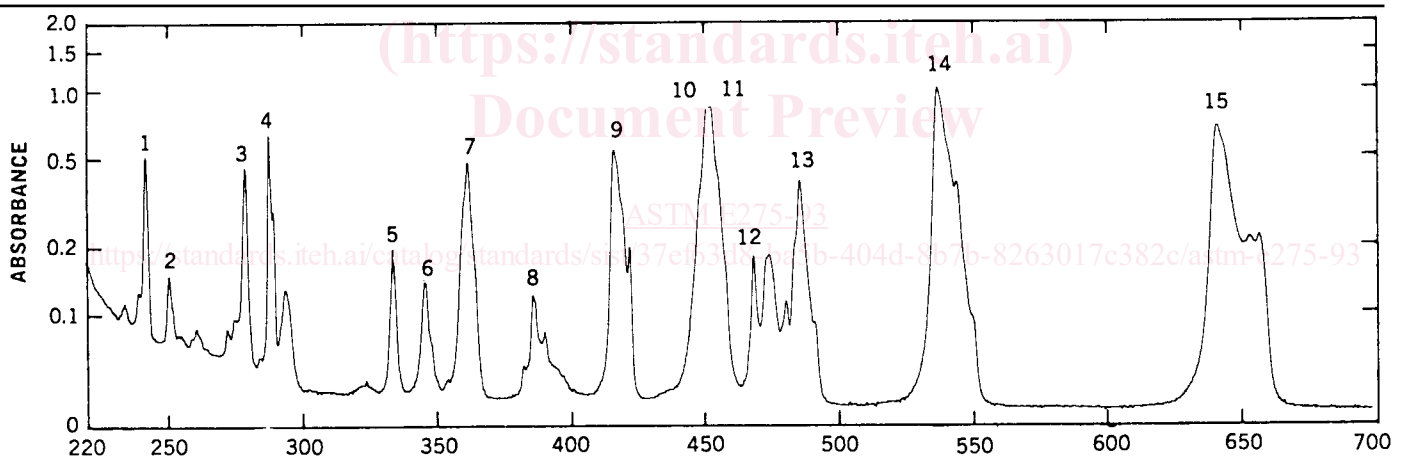


WAVELENGTH, nm							
Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm	Band Number	Wavelength, nm
3	279.4	7	360.9	10	453.2	14	536.2
4	287.5	8	385.9	12	460.0	15	637.5
5	333.7	9	418.7	13	484.5		

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 (Corning No. 3130) Showing Reference Wavelength<sup>10</sup>



WAVELENGTH, nm							
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

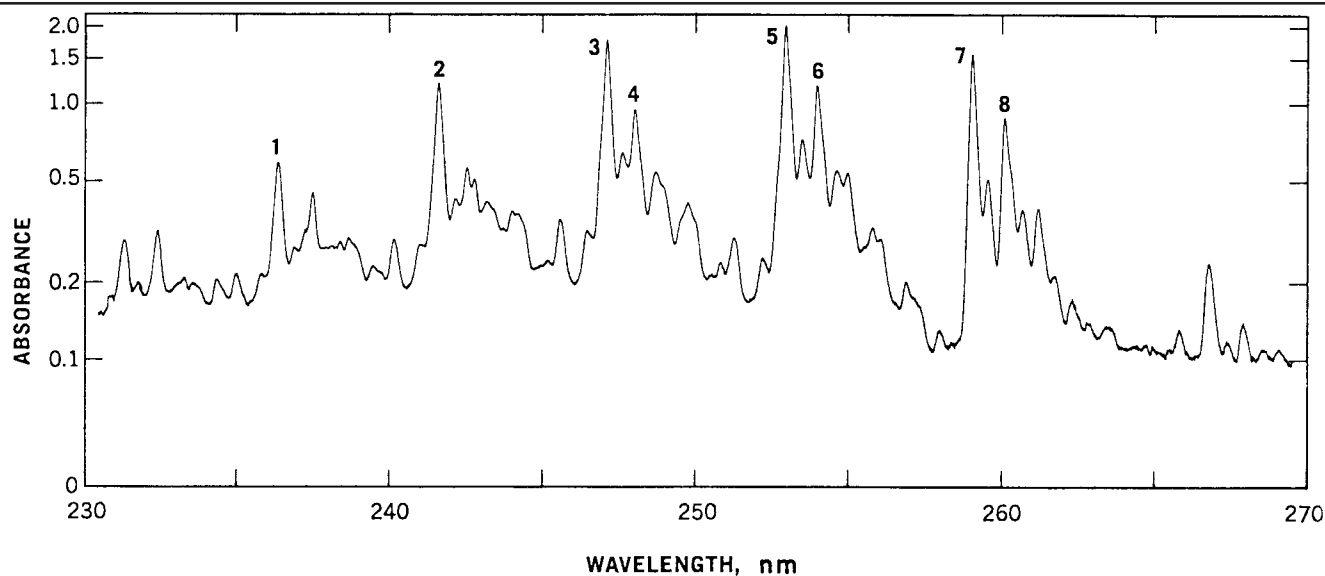
FIG. 3 Spectrum of 4 Percent Solution of Holmium Oxide in 1.4 M Perchloric Acid (1.00-cm Cell) Showing Reference Wavelengths<sup>10</sup>

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<sup>12</sup>

### 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 glass.<sup>7</sup> 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), or the absorption spectrum of 1,2,4-trichlorobenzene (Fig. 6).

12.2 The high-pressure mercury arc spectrum<sup>8,9,10</sup> 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.

### 13. Measurement Procedure

13.1 *Measurement Procedure for Monochromator-Based (Non-Digital) Spectrophotometers:*

<sup>7</sup> The National Institute of Standards and Technology supplies didymium glass filters as SRM 2009a. (Detailed information on these filters is presented in Ref 2).

<sup>8</sup> The General Electric H100-A4 lamp has been found satisfactory for this purpose.

<sup>9</sup> *Handbook of Chemistry and Physics*, 74th Ed. (1993), Chemical Rubber Publishing Co., Cleveland, Ohio.

<sup>10</sup> McNeirney, J., and Slavin, W., *Applied Optics*, APOPA, Vol 1, 1962, p. 365.

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:

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

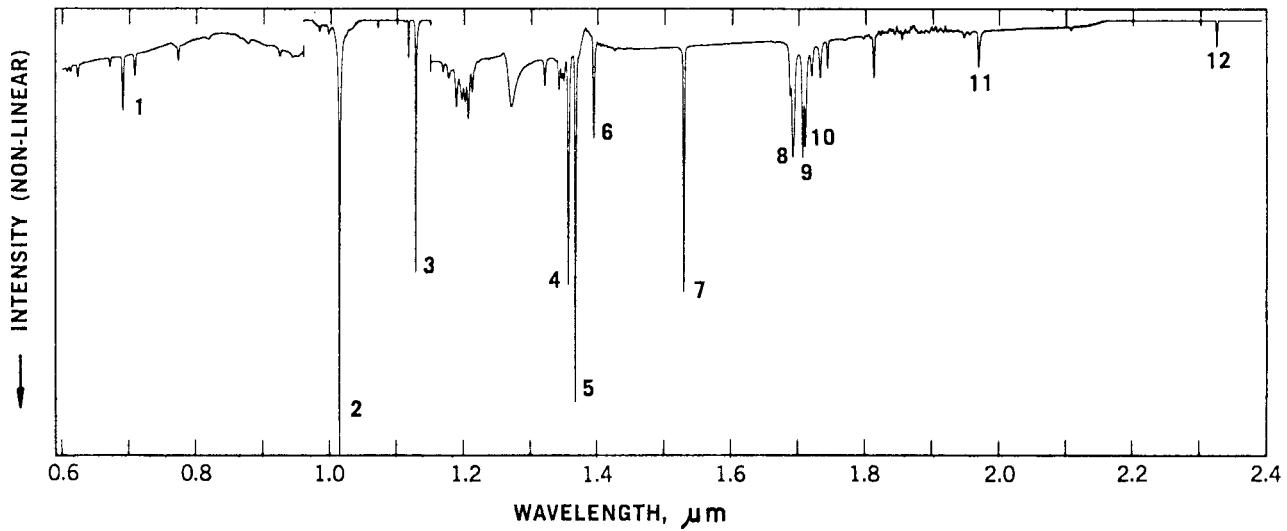
where:

- S = standard deviation,
- $\lambda_i$  = individual observed wavelength,
- $\lambda_{aver}$  = averaged observed wavelength, and
- n = number of observations (in this case, n = 10).

13.2 *Measurement Procedure for Diode Array Spectrophotometers:*

13.2.1 Prevent light from reaching the detector and measure the dark signal of each diode. A convenient number of measurements (10) is made to allow the standard deviation of the dark current to be determined. The reference lamp (low-pressure Mercury) is placed into the sample compartment, and turned on, and the light is allowed to reach the detector. Select





Line	Wavelength ( $\lambda$ ), $\mu\text{m}$	Line	Wavelength ( $\lambda$ ), $\mu\text{m}$	Line	Wavelength ( $\lambda$ ), $\mu\text{m}$	Line	Wavelength ( $\lambda$ ), $\mu\text{m}$
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

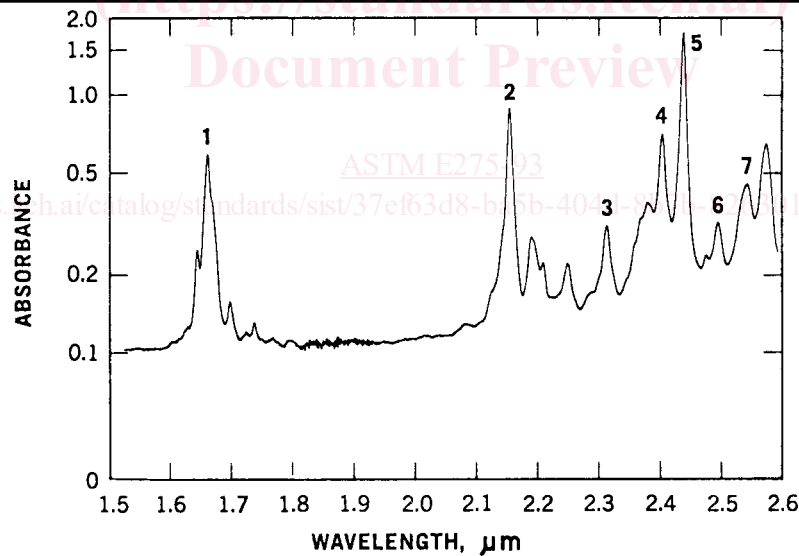
Slit Width: 0.2 mm for Lines 2 and 3;

0.5 mm for other lines

Spectral Slit Width: 0.001  $\mu\text{m}$  for Lines 2 and 3;

0.002  $\mu\text{m}$  for other lines

FIG. 5 Emission Spectrum of High-Pressure Mercury Arc Showing Reference Wavelengths<sup>9</sup>



Band Number	Wavelength, $\mu\text{m}$	Band Number	Wavelength, $\mu\text{m}$	Band Number	Wavelength, $\mu\text{m}$	Band Number	Wavelength, $\mu\text{m}$
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  $\mu\text{m}$

Cell Thickness: 0.1 cm

FIG. 6 Spectrum of 1, 2, 4-Trichlorobenzene Showing Reference Wavelengths in the Near-Infrared Region<sup>14</sup>

two calibration wavelengths preferably bracketing the analytical wavelength. Measure the wavelength of the selected emission lines ten times and calculate the average. The wavelength accuracy is the difference between the true wavelength and the average observed reading.

13.2.2 A more accurate estimate of wavelength accuracy for diode array devices can be determined by calculating the true center wavelength for each diode by using measurements of the intensity of the emission line at all corresponding diodes and then calculate the shift in wavelength from the expected, taking