Designation: E 169 – 99

Standard Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis[1]

This standard is issued under the fixed designation E 169; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These practices are intended to provide general information on the techniques most often used in ultraviolet and visible quantitative analysis. The purpose is to render unnecessary the repetition of these descriptions of techniques in individual methods for quantitative analysis.

1.2 *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:*

E 131 Terminology Relating to Molecular Spectroscopy[2]

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

E 275 Practice for Describing and Measuring the Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers[2]

E 925 Practice for Periodic Calibration of Narrow Band-Pass Spectrophotometers[2]

E 958 Practice for Measuring Practical Spectral Bandwidth of Ultraviolet-Visible Spectrophotometers[2]

3. Summary of Practice

3.1 Quantitative ultraviolet and visible analyses are based upon the absorption law, known as Beer's law. The units of this law are defined in Terminology E 131. Beer's law (Note 1) holds at a single wavelength and when applied to a single component sample it may be expressed in the following form (see Section 10):

$$A = abc \tag{1}$$

When applied to a mixture of *n* noninteracting components, it may be expressed as follows:

$$A = a_1bc_1 + a_2bc_2 + \ldots. + a_nbc_n \tag{2}$$

NOTE 1—Detailed discussion of the origin and validity of Beer's law may be found in the books and articles listed in the bibliography at the end of these practices.

3.2 This practice describes the application of Beer's law in typical spectrophotometric analytical applications. It also describes operating parameters that must be considered when using these techniques.

4. Significance and Use

4.1 These practices are a source of general information on the techniques of ultraviolet and visible quantitative analyses. They provide the user with background information that should help ensure the reliability of spectrophotometric measurements.

4.2 These practices are not intended as a substitute for a thorough understanding of any particular analytical method. It is the responsibility of the user to familiarize him or herself with the critical details of a method and the proper operation of the available instrumentation.

5. Sample Preparation

5.1 Accurately weigh the specified amount of the sample (solid or liquid). Dissolve in the appropriate solvent and dilute to the specified volume in volumetric glassware of the required accuracy. (Solvent and flask should be approximately the same temperature as the spectrophotometer). If needed, a dilution should be made with a calibrated pipet and volumetric flask, using adequate volumes for accuracy. Fill the absorption cell with the solution, and fill the comparison or blank cell with the pure solvent.

6. Cell and Base-Line Checks

6.1 Clean and match the cells. Suggested cleaning procedures are presented in Practice E 275.

6.2 Establish the base-line of a recording double-beam spectrophotometer by scanning over the appropriate wavelength region with pure solvent in both cells. Determine apparent absorbance of the sample cell at each wavelength of interest. These absorbances are cell corrections that are subtracted from the absorbance of the sample solution at the corresponding wavelengths.

[1] These practices are under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and are the direct responsibility of Subcommittee E13.01on Ultraviolet and Visible Spectroscopy.

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

6.3 For single beam instruments, either use the same cell for pure solvent and sample measurements, use matched cells, or apply appropriate cell corrections.

6.4 For some newer instruments, the cell corrections or the blank cell absorbance is stored in memory and automatically incorporated into the sample absorbance measurement.

6.5 An accurate determination of cell path length in the 1-cm range is not practical in most laboratories, and common practice is to purchase cells of known path length.[3] A check on path length matching, however, may be made by measuring the absorbance of a strongly absorbing solution (A~0.9) versus pure solvent, and then emptying, cleaning, reloading fresh solvent and sample into the other cells, and remeasuring the absorbance. Similarly, the absorbance of the sample in a series of cells to be used in an analysis can be measured versus pure solvent in a given reference cell.

7. Analytical Wavelengths and Photometry

7.1 Analytical wavelengths are those wavelengths at which absorbance readings are taken for use in calculations. These may include readings taken for purposes of background corrections. The analytical wavelengths are frequently chosen at absorption maxima, but this is not always necessary. For example, the use of isoabsorptive or isosbestic points is frequently useful.

7.2 Record the absorbance readings at the specified analytical wavelengths, operating the instrument in accordance with the recommendations of the manufacturer or Practice E 275.

7.3 Absorbance values should be used only if they fall within the acceptably accurate range of the particular spectrophotometer and method employed. If the absorbance is too low, either use a longer absorption cell or prepare a new solution of higher concentration. If the absorbance is too high, use a shorter cell or make a quantitative dilution. If different cells are used, a new base-line must be obtained.

7.4 The precision and bias of the wavelength and photometric scales of the instrument must be adequate for the method being used. Procedures for checking precision and accuracy of these scales are presented in Practices E 275 and E 925.

8. Spectral Band Width and Slit Width

8.1 If the analytical method specifies a spectral band width or a spectral slit width, set the spectral band width of the instrument to the specified value. If the instrument has only a mechanical slit width indicator, use the information provided in the manufacturer's literature to calculate the slit width that corresponds to the specified spectral band width.

NOTE 2—The accuracy of spectral band width and mechanical slit width indicators can be determined using the procedure given in Practice E 958.

8.2 If the analytical method specifies a mechanical slit width for a particular type of instrument and the same type of instrument is being used, set the slit width to the specified values. If a different type of instrument is being used and information is available from which the spectral slit width of both types of instruments can be calculated, adjust the instrument settings to obtain a spectral slit width equal to the one calculated from the specified mechanical slit width.

8.3 If the analytical method does not state a spectral band width or a slit width value but includes a spectrum illustrating adequate resolution, set the spectral band width or slit width of the instrument to obtain comparable resolution.

8.4 If the method neither specifies spectral band width or slit width nor provides an illustrative spectrum, use the smallest spectral band width or slit width that yields an acceptable signal-to-noise ratio. Record this value for future reference.

NOTE 3—Changes in the day-to-day values of spectral band width or slit width obtained with a given gain, or changes in gain required to obtain a given spectral band width or slit width, are indicative of present or potential problems. Increased spectral band width or gain may result from a lower output of the light source, deterioration of optical components, deposits on the windows of the cell compartment or on the inside wall of the reference cell, an absorbing impurity in the solvent, or a faulty electronic component.

9. Solvents and Solvent Effects

9.1 The ultraviolet absorption spectrum of a compound will vary in different solvents depending on the chemical structures involved. Nonpolar solvents have the least effect on the absorption spectrum. Nonpolar molecules in most instances are not affected in polar solvents. However, polar molecules in polar solvents may show marked differences in their spectra. Any interaction between solute and solvents leads to a broadening and change in structural resolution of the absorption bands. Ionic forms may be created in acidic or basic solutions. In addition, there are possible chemical reactions between solute and solvent, and also photochemical reactions arising from either room illumination or the short wavelengths in the beam of the spectrophotometer. It is important that the solvent used be specified in recording spectral data. (The change in spectra between acidic and basic conditions may sometimes be employed in multicomponent analysis.)

9.2 Common commercially available solvents of "spectroscopic purity" are listed in Table 1. The short wavelength limit

TABLE 1 Solvents[A]

Solvent	Cutoff, nm
Pyridine	305
Tetrachloroethylene	290
Benzene	280
N,N-Dimethylformamide	270
Carbon tetrachloride	265
Methyl formate	260
Chloroform	245
Dichloromethane	235
Ethyl ether	220
Acetonitrile	215
Isopropyl alcohol	210
Ethyl alcohol	210
Methyl alcohol	210
Cyclohexane	<210
*Iso*octane	<210

[A] Procedures for special purification of solvents for further improvement in the wavelength limit are given in Refs **(11, 12)**. Solvents of high purity for use in absorption spectroscopy also are available commercially.

Spectranalyzed[tm] solvents, Fisher Scientific, 711 Forbes Ave, Pittsburgh, PA 15219

Spectroquality Solvents, Matheson, Coleman and Bell, 11-38 31st Ave., Long Island City, NY 11106

[3] Cells having a 1-cm pathlength specified to an accuracy of ±0.01 mm are available from a number of manufacturers.

is approximate, and refers to the wavelength at which a 1-cm light path length gives an absorbance of unity.

9.3 Water, and 0.1 *N* solutions of hydrochloric acid, sulfuric acid, and sodium hydroxide also are commonly used as solvents. Buffered solutions, involving nonabsorbing materials, are frequently used; both the composition of the buffer and the measured pH should be specified. Mixtures of 0.1 *N* dihydrogen sodium phosphate and 0.1 *N* hydrogen disodium phosphate are useful in the 4.5 to 8.9 pH range. A table of nonabsorbing buffers has been presented by Abbott **(10)**.[4]

10. Calculations

10.1 Quantitative analysis by ultraviolet spectrophotometry depends upon Beer's law. The terms and symbols used are those defined in Terminology E 131. According to Beer's law:

$$A = abc = (\epsilon/M) \times bc \tag{3}$$

where:
A = absorbance,
a = absorptivity,
b = cell length, cm,
c = concentration, g/L,
ϵ = molar absorptivity, and
M = molecular weight.

10.1.1 In practice, a distinction must be made between c, the concentration of the absorbing material in the cell at the time of observation, and the concentration of the *absorbing material* in the *sample as received.* This is here designated as a mass fraction C (g/g). The solution to be examined has a concentration of *sample* in solution, C_s, which is in units of grams per litre.

$$c = A/ab \tag{4}$$

$$C = c/C_s = A/(abC_s) \tag{5}$$

10.2 If one or more dilutions are then made, the quantity called the dilution factor must be included. Dilution factor, f, is the ratio of the final volume to the initial volume. If more than one dilution is performed, the dilution factor is the product of the factors from each dilution. If dilutions are made, the equation becomes the following:

$$C = cf/C_s = Af/(abC_s) \tag{6}$$

Note that c and C_s, have the dimensions of grams per litre. If dilution is made, $C_s$is not the concentration in the cell at the time the absorbance is determined; the concentration in the cell is C_s/ f.

10.3 *Chemical Calibration*—The absorptivity of the absorbing material, the concentration of which it is desired to determine, is obtained by examination of a series of quantitative dilutions of a neat sample of this material. However, if no such neat sample is available, the best available material is used, or a value of the absorptivity is taken from the literature. Take care to specify this, by reporting values as "percentage against calibration material" or by noting that the accuracy of the analysis is dependent upon a published value of the absorptivity or molar absorptivity. (A reference must be cited.)

10.3.1 Some sample materials are highly fluorescent which can significantly reduce the measured absorbance. The effect of sample fluorescence may vary depending upon the spectrophotometer and wavelength chosen. Sample fluorescence may be a particular problem when using published absorptivity values.

10.4 *Types of Analyses* (see Fig. 1):

10.4.1 *One Component, No Background Correction*:

$$C = Af/(abC_s) \tag{7}$$

10.4.2 *One Component, Simple Background Correction*:

$$C = \frac{(A_1 - A_2) \times f}{a_1 b C_s} \tag{8}$$

where the subscripts refer to analytical wavelengths. The term A_2 is the absorbance at the wavelength used for making a simple subtractive correction. It is usually selected from examination of the spectral curve of the reference material at a wavelength longer than that of A_1, preferably where a_2 is much less than a_1.

10.4.3 *One Component, with Slope-Type Background Correction*:

$$C = \frac{[A_1 - A_2 + S(\lambda_2 - \lambda_1)]\, f}{a_1 b C_s} \tag{9}$$

where:
S = slope between wavelengths 1 and 2 for the background.

10.4.3.1 The background absorption is usually *not* linear between the analytical wavelength and the wavelength at which a simple subtractive background correction may be obtained. When it is possible to determine the slope between wavelengths 1 and 2 by observation of the samples that do not contain the absorbing material that is to be determined, this may be used as a correction for the background absorption.

10.4.4 *One Component, With Linear Background Correction*:

10.4.4.1 The equation for the general case is as follows:

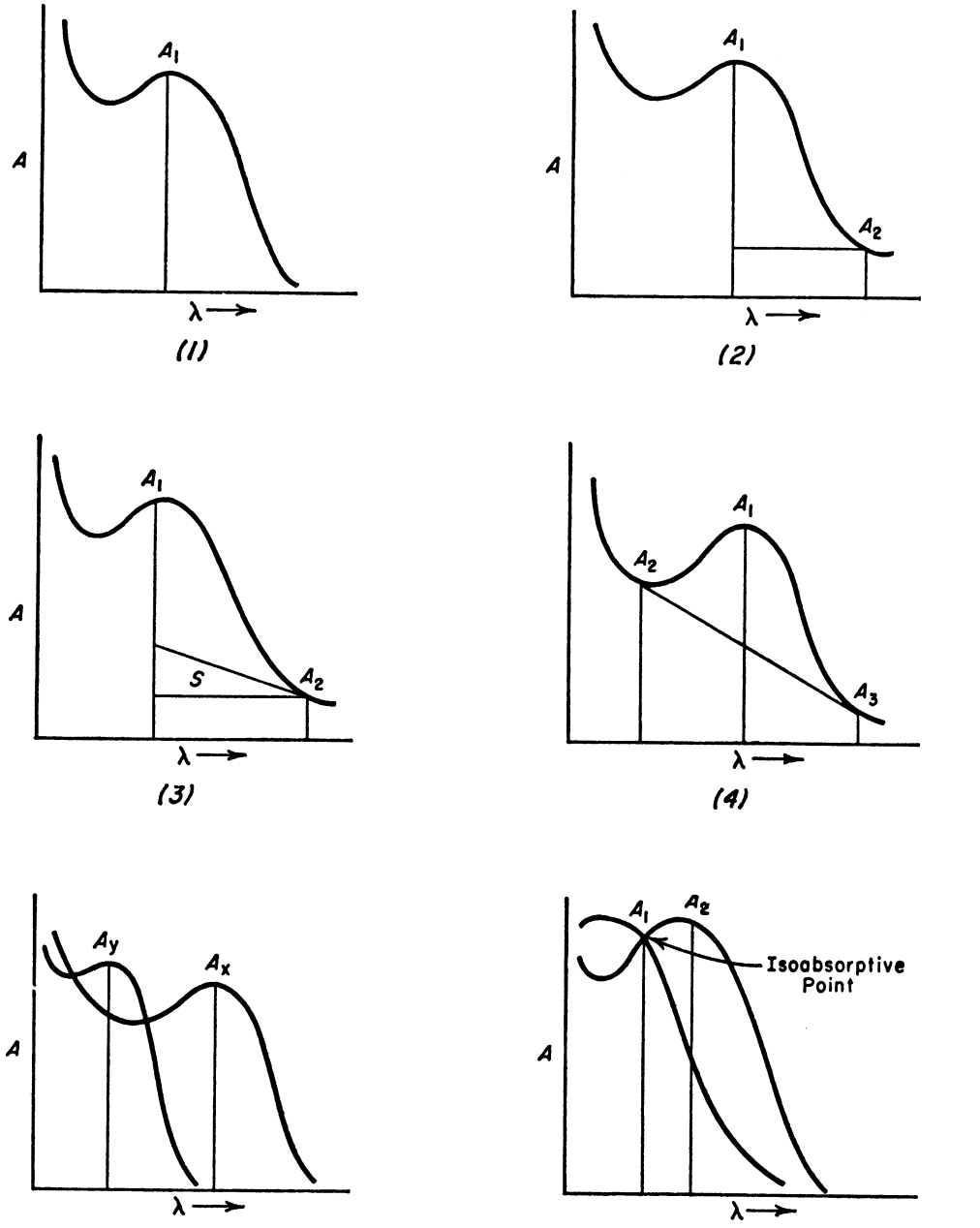
$$C = \frac{A_1 - \left[A_3 + [A_2 - A_3] \times \frac{\lambda_3 - \lambda_1}{\lambda_3 - \lambda_2}\right] f}{abC_c} \tag{10}$$

The absorptivity a is here the effective absorptivity as determined on a pure sample, using the corrections, and is somewhat lower than the true or absolute absorptivity.

10.4.4.2 This method is especially effective with materials that have sharp bands. Wavelengths 2 and 3 are selected to the long and short wavelength sides of the analytical wavelength 1, usually at absorbance minima.

10.4.4.3 For the special case where the wavelength for A_1 is exactly midway between the wavelengths for A_1 and A_3, the equation reduces to the following:

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



(1) One Component, No Background Correction.
(2) One Component, Simple Background Correction.
(3) One Component, with Slope-Type Background Correction.
(4) One Component, with Linear Background Correction.
(5) Two Components, with Overlapping Absorption for Only One Component.
(6) Two Components, with Mutually Overlapping Absorption.

FIG. 1 Plot of Components

$$C = f\left[\frac{A_1 - \frac{A_2 + A_3}{2}}{abC_s}\right] \quad (11)$$

10.4.5 *One Component, With Background Correction from Outside Data*:

$$C = \frac{(A - X) \times f}{abC_s} \quad (12)$$

where X is a background absorbance calculated from outside data.

10.4.5.1 This is a general case in which some empirical correction may be derived from data other than spectrophotometric, and is applied as an effective absorbance which is subtracted from the observed. As an example, the concentration of a known interfering material may be determined by titration, and the absorbance due to this calculated, and then subtracted.

10.4.6 *Two Components, With No Overlapping Absorption*—Apply the method in 10.4.1 twice, at the two analytical wavelengths. This is an almost impossible case, except when the relative concentrations of the two components are such that the product of absorptivity and concentration of

one component at a given wavelength is more than 100 times the product for the other component, allowing the latter to be neglected.

10.4.7 *Two Components, With Overlapping Absorption for Only One Component*—Determine the component with no interference (component *x*) at an analytical wavelength, λ_1, selected to allow no contribution from component *y* as follows:

$$C_x = fA_1\,(a_{1x}bC_s) \tag{13}$$

10.4.7.1 Calculate the contribution of this component to the observed absorbance at the other analytical wavelength, λ_2, where both components are absorbing, as follows:

$$A_{2x} = a_{2x}bc_x = a_{2x}bC_xC_s/f \tag{14}$$

10.4.7.2 Calculate the concentration of component *y* as follows:

$$C_y = [(A_2 - A_{2x}) \times f\,]\,/a_{2y}bC_s \tag{15}$$

10.4.8 *Two Components, with Mutually Overlapping Absorption*—Use the absorbance-ratio method (graphical) described in Ref **(13)** or by simultaneous equations as follows:

$$C_x = \frac{[a_{2x}A_1 - a_{1y}A_2]\,f}{bC_s \times (a_{2y}a_{1x} - a_{1y}a_{2x})} \tag{16}$$

$$C_y = \frac{[a_{2x}A_1 - a_{1a}A_2]\,f}{bC_s \times (a_{1y}a_{2x} - a_{2y}a_{1x})} \tag{17}$$

10.4.9 *Inverted Matrix Method, for Two or More Components, With Mutually Overlapping Absorption*—For information on the inverted matrix method, see Sections 10 and 17 of Practices E 168.

10.5 *Computerized Calculations*—Newer instruments may perform automatically many of the calculations described in 10.4. The user should be aware of the algorithms used by the manufacturer. It is recommended that the user verify the reliability of computed results by periodically performing the calculations using the raw analytical data.

11. Presentation of Data

11.1 If absorption curves are to be presented with an analytical method, it is recommended that one of the following systems be used, with the wavelength (in nanometers) increasing linearly to the right:

$\log \epsilon$ or $\log a$ plotted against λ

A plotted against λ

$\epsilon \times 10^{-n}$ or a plotted against λ

where the symbols are as defined in Terminology E 131. Marking the analytical wavelengths and absorptivity values on the curve is suggested for clarity, or a separate table of analytical wavelengths and absorptivities may be used. (These data are helpful for others who may wish to use the method in a somewhat modified form.)

12. Keywords

12.1 molecular spectroscopy; quantitative analysis

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