Designation: E169 – 16

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

This standard is issued under the fixed designation E169; 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 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

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

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

E925 Practice for Monitoring the Calibration of Ultraviolet-Visible Spectrophotometers whose Spectral Bandwidth does not Exceed 2 nm

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

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 E131. 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 11):

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

When applied to a mixture of n non-interacting 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 users to familiarize themselves 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, ensuring that all appropriate temperature range tolerances are maintained. If needed, a dilution should be made with a calibrated pipet and volumetric flask, using adequate volumes for accuracy. With the availability of moderin wide

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[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.

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range electronic balances, (capable of reading kg quantities to four or five decimal places), gravimetric dilution should be considered as a more accurate alternative to volumetric, if available. Fill the absorption cell with the solution, and fill the comparison or blank cell with the pure solvent, at least 2× to 3× (if sufficient sample or solvent is available), before measuring.

5.2 The solution should be visibly clear, and free from particulate matter. However, there may still be present suspended particles not visible to the naked eye, and these will still scatter light by the Tyndall effect, causing a decrease in the measured intensity that increases as the wavelength decreases. Unless there is no alternative, absorbance should not be determined on turbid or light scattering samples. Any measurements performed on a light scattering solution are highly instrument specific and can be used only for comparative purposes in the same system.

NOTE 2—To avoid the dilution step, the instrument may contain an automatic system which will allow adjustment of the path length of the measurement cell to optimize the measured absorbance.

6. Cell and Base-Line Checks

6.1 Clean and match the cells. Suggested cleaning procedures are presented in Practice E275.

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.

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 On most software-controlled 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. Modern cell manufacturing techniques employed by a number of leading manufacturers can guarantee the path length of a 1-cm cell to ±0.01 mm or better.

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. To minimize the effect of wavelength error, the analytical wavelengths are frequently chosen at absorption maxima, but this is not always necessary. If the wavelength accuracy of the spectrophotometer is such that the calculated uncertainty in the absorbance measurement is within acceptable limits at the extremes of this wavelength uncertainty range, then single point measurements on a slope can be used. For example, the use of isoabsorptive or isosbestic points is frequently useful.

NOTE 3—If the sample matrix includes fluorescent compounds, the measured signal usually will contain a contribution from fluorescence.

7.2 Record the absorbance readings at the specified analytical wavelengths, operating the instrument in accordance with the recommendations of the manufacturer or Practice E275.

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.[3] 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 E275 and E925.

8. Stray Radiant Energy (Stray Light)

8.1 The acceptable absorbance range for any given instrument will be governed not only by the specification of the spectrophotometer, but also by the condition at time of measurement.

8.2 Given that the measurement is fundamentally a difference in energy in an optical system, the factors affecting the measurement may include, but not be limited to: the output from the source(s), efficiency of the grating, cleanliness of the mirrors, etc.

8.3 Stray radiant energy in any instrument system may begin to cause a negative deviation error, long before the transmittance (absorbance) limit is reached. An effective estimation may be performed using Practice E387.

9. Resolution and Bandwidth

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

NOTE 4—The accuracy of resolution and mechanical bandwidth indicators can be determined using the procedure given in Practice E958.

9.2 If the analytical method does not state a required resolution or a bandwidth value but includes an illustrative spectrum, set the resolution or bandwidth of the instrument to obtain comparable data. As a rule of thumb, the resolution should be less than one-eighth of the bandwidth; thus for a peak of bandwidth 40 nm, the resolution should not exceed 5 nm.

9.3 If the method neither specifies resolution or bandwidth nor provides an illustrative spectrum, use the smallest resolution or bandwidth that yields an acceptable signal-to-noise ratio. Record this value for future reference.

[3] The errors associated with cell path lengths are significantly less than those generated by volumetric dilution, and therefore where possible, different path length cells should be used in preference to volumetric procedures.