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Standard Practice for the Periodic Calibration of Narrow Band-Pass Spectrophotometers¹

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^{ε1} NOTE—Section 11 was added editorially in June 1994.

INTRODUCTION

In the application of spectrophotometric methods of analysis it is the responsibility of the analyst to verify and validate that the instrument is functioning properly and is capable of providing acceptable analytical results. It is preferable that the verification of instrument performance be accomplished through the use of readily available, stable reference materials whose properties have been accurately measured by a standardizing laboratory. Several such certified standards are currently available from the National Bureau of Standards and their use in the tests and measurements described in this practice is satisfactory for evaluating the performance of spectrophotometers.

This practice covers some of the essential instrumental parameters that should be evaluated to ensure the acceptability of the analytical data routinely obtained on the instrument. These parameters include the accuracy of the wavelength and absorbance scales and stray radiant power levels.

The accuracy of the wavelength scale in both the UV and VIS regions is determined using the sharp absorption bands of a holmium oxide glass filter.² The wavelength of maximum absorbance of these bands has been determined.³ The accuracy of the absorbance scale is determined using certified standards available through the National Bureau of Standards. The absorbance scale accuracy in the UV region (235–350 nm) is determined using acidic solutions of potassium dichromate (solid potassium dichromate is available from the National Bureau of Standards as SRM 935) while in the visible region (440–635 nm) the absorbance accuracy is determined using three glass filters (National Bureau of Standards SRM 930 series).⁴ The use of these reference materials provides a valid and relatively simple means to test the accuracy of the wavelength and absorbance scales of narrow band-pass spectrophotometers in the spectral ranges indicated.

1. Scope

1.1 This practice covers the parameters of spectrophotometric performance that are critical for testing the adequacy of instrumentation for most routine tests and methods.⁵ The recommended tests provide a measurement of the important parameters controlling results in spectrophotometric methods, but it is specifically not to be inferred that all factors in instrument performance are measured.

1.2 This practice may be used as a significant test of the

performance of instrumentation. While this procedure is not designed to extensively evaluate the performance of an instrument, it may be used to verify quantitative performance on an ongoing basis and to compare one instrument's performance with that of other units.

1.3 This practice should be performed on a periodic basis, the frequency of which depends on the physical environment within which the instrumentation is used. Thus, units handled roughly or used under adverse conditions (exposed to dust, chemical vapors, vibrations, or combinations thereof) should be calibrated more frequently than those not exposed to such conditions. This practice should also be performed after any significant repairs are made on a unit, such as those involving the optics, detector or radiant energy source.

1.4 *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.*

¹ 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.

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² Available from Beckman Instruments (Part No. 96157) and from distributors from Corning Glass Works (Catalogue No. 3131).

³ McNeirney, J., and Slavin, W., *Applied Optics*, Vol 1, 1962, p. 365.

⁴ SRM 935 and 930 may be purchased from the Office of Standard Reference Materials, National Bureau of Standards, Washington, D. C. 20234.

⁵ Routine tests are defined as those in which absorbance data obtained on a sample are compared to those of a standard sample preparation.

2. Referenced Documents

2.1 ASTM Standards:

- E 131 Terminology Relating to Molecular Spectroscopy⁶
- E 169 Practices for General Techniques of Ultraviolet/Visible Quantitative Analysis⁶
- E 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near Infrared Spectrophotometers⁶

2.2 Other Publication:

- NBS Special Publication 260-54: Certification and Use of Acidic Potassium Dichromate Solutions As An Ultraviolet Absorbance Standard⁷

3. Terminology

3.1 Definitions:

- 3.1.1 For the definitions of terms used in this practice, refer to Terminology E 131.
- 3.1.2 For a description of the instrumental parameters evaluated in this practice, refer to Practice E 275.
- 3.1.3 For a description of quantitative ultraviolet spectrophotometric techniques, refer to Practice E 169.

4. Significance and Use

4.1 This practice permits an analyst to compare the performance of an instrument to the manufacturer’s supplied performance specifications and to verify its suitability for continued routine use. It also provides generation of calibration data on a periodic basis, forming a base from which any changes in the performance of the instrument will be evident.

5. Reference to this Calibration Procedure

5.1 Reference to this practice in any spectrophotometric calibration scheme shall constitute due notification that the adequacy of the spectrophotometer performance has been evaluated by means of this practice. Performance is considered to be adequate when the calibration data obtained are in agreement with the true values.

6. Instrument Operation

- 6.1 In obtaining spectrophotometric calibration data the analyst must select the proper instrumental operating conditions to realize satisfactory instrument performance. Operating conditions for individual instruments are best obtained from the manufacturer’s literature because of variations in instrument design.
- 6.2 When using calibration standards, all the components of the spectrophotometer must be functioning properly. In addition, the temperature of the specimen compartment should be between 23° and 30°C. Matched solution cells should be used for calibration purposes.
- 6.3 Each of the above factors in instrument operation is important in the determination of wavelength and absorbance accuracy.

7. Determination of Wavelength Accuracy in the Ultraviolet and Visible Spectral Regions

7.1 Discussion—The holmium oxide glass filter is used for evaluating wavelength accuracy. The wavelengths (nm) monitored are:

- 279.4
- 287.5
- 360.9
- 418.7
- 453.2
- 536.2
- 637.5

NOTE 1—Depending on the base glass of the filter, a peak at 241.5 nm may also be used for calibration purposes.

7.1.1 If the wavelength scale is found to be less accurate than the tolerance stated in the instrument manufacturer’s specifications, or if the observed absorption bands of the holmium oxide glass deviate by more than ±1 nm from the values stated above (see Note 2) then corrective service must be performed on the instrument by a qualified service person. If the user performs this service himself, he should carefully follow the manufacturer’s recommended procedure.

NOTE 2—The larger of these tolerance values should be used as the criterion of instrumental wavelength accuracy at each wavelength checked.

7.1.2 The wavelength accuracy is dependent on the spectral bandwidth and thus on the slit width. Spectral bandwidths may be determined from the manufacturer’s specifications.

7.2 Procedure:

- 7.2.1 Examine the holmium oxide filter and remove any surface contamination using a soft brush or lens paper. Measure the temperature of the sample compartment by placing a thermometer into the cell compartment of a stabilized instrument and replacing the compartment cover securely. Place the thermometer bulb as close to the actual position that will be occupied by the standard. After a suitable period of time record the temperature reading, remove the thermometer, and resume normal operations.
- 7.2.2 Record the blank absorbance (air versus air) readings at the appropriate wavelength intervals and scan speeds and perform any necessary baseline adjustments. Record the wavelength of maximum absorbance as a function of wavelength at each appropriate wavelength interval and scan speed. If large discrepancies (>1 nm) exist between the true and measured wavelengths, repeat the procedure at a slower scan speed and narrower slit width if possible to verify the nonconformity.
- 7.2.3 Report the wavelength calibration data in Table 1.

8. Evaluation of Stray Radiant Power Ratio (SRPR)

8.1 Discussion—A portion of the unwanted stray radiant power detected by the photodetector can be measured using the following sharp cut-off solution filters in 1-cm cells:

| Solution | Wavelength |
|--------------------------------------------------------------------------------|------------|
| KI, 1.0 g/L in H ₂ O | 220 nm |
| K ₂ Cr ₂ O ₇ , 0.25 g/L in aqueous 0.05 N KOH | 370 nm |

8.1.1 Reagent grade materials should be used for these

⁶ Annual Book of ASTM Standards, Vol 03.06.
⁷ Available from the National Technical Information Service, Port Royal Road, Springfield, VA 22161.

TABLE 1 UV-VIS Spectrophotometers Wavelength and Stray Radiant Power Ratio Calibration

Instrument _____
 Date _____
 Temperature _____
 Analyst _____

Wavelength Calibration: Holmium Oxide Filter

| True Wavelength (nm) Range | Observed Wavelength (nm) | Scan Speed | Wavelength Tolerance | |
|----------------------------|--------------------------|------------|----------------------|------------------|
| | | | Conforms | Does Not Conform |
| 278.4–280.4 | | | | |
| 286.5–288.5 | | | | |
| 359.9–361.9 | | | | |
| 417.7–419.7 | | | | |
| 452.2–454.2 | | | | |
| 535.2–537.2 | | | | |
| 636.5–638.5 | | | | |

Stray Radiant Power Ratio

| Wavelength (nm) | Transmittance or Absorbance | Conforms | Does Not Conform |
|-----------------|-----------------------------|----------|------------------|
| 220 | | | |
| 370 | | | |

solutions. They are essentially opaque at the indicated wavelengths; any observed transmittance is equivalent to the SRPR. However, it should be recognized that these solutions absorb most of the stray radiation within 40 nm of the indicated wavelengths and a significant fraction of still more distant stray radiation. Consequently the tests underestimate the true SRPR. The underestimation may be severe in instruments using relatively narrow-band blocking filters.

8.1.2 An acceptable level of SRPR depends on the spectral character and absorbance level of the sample under investigation. Whenever the SRPR exceeds the manufacturer’s specification the instrument should be serviced. An excessive SRPR usually arises from dust, scratches, or corrosion on the collimator or disperser, or both. Correction of this problem should be performed by qualified personnel. Care should be taken to discriminate between SRPR and light leaks. The latter most often originate in the sample compartment and can be detected by blocking the sample beam alternately at the ports on the source and detector sides of the sample compartment. Any difference in the detected signals indicates a light leak.

8.1.3 While the stray radiant power ratio is equivalent to the transmittance described previously, it is often preferable to make the measurement in the absorbance mode and mathematically convert absorbance to transmittance.

8.2 Procedure:

8.2.1 Use the visible light source lamp in the 370 nm region and the ultraviolet light source lamp in the 220 nm region.

8.2.2 Determine the transmittance or absorbance of each solution at the appropriate wavelength using the indicated solvents for reference.

8.2.3 In Table 1, report the transmittance or absorbance of these solutions. Note whether the measured stray radiant power ratio exceeds the manufacturer’s specification.

9. Determination of the Absorbance Scale Accuracy in the Ultraviolet and Visible Spectral Regions

9.1 *Discussion*—The accuracy of the absorbance scale is determined using certified standards available through the

National Bureau of Standards. The absorbance scale accuracy in the ultraviolet region (235 to 350 nm) is determined using acidic solutions of potassium dichromate (National Bureau of Standards SRM 935) while in the visible region (440 to 635 nm) the absorbance accuracy is determined using three essentially neutral density glass filters (National Bureau of Standards SRM 930 series).

9.1.1 If the measured absorbances (^Ameasured) of the standards are outside the acceptable range, then corrective service must be performed on the instrument by a qualified service person. If the user performs this service himself, then he should carefully follow the manufacturer’s recommended procedure.

9.1.2 An acceptable absorbance range for each standard for any instrument must be determined based on the instrument manufacturer’s specifications. As a guide to the acceptability of photometric accuracy data a tolerance of ±1.5 % is recommended and is based on the random errors inherent in the preparation and use of these standards.

9.1.3 The data obtained during the calibration of the absorbance accuracy in both the visible and ultraviolet regions can be used to construct absorbance scale correction curves. The absorbance scale correction curve is useful in high accuracy work when an analyst needs to convert a measured absorbance reading into a corrected absorbance reading. Rigorous treatment of the construction and use of this correction curve is beyond the scope of this practice but the generation and use of such a calibration curve is described in the NBS special publication 260-54.

9.1.4 Studies by the NBS have indicated that properly prepared and stored solutions of SRM 935 are stable for at least six months. Series of SRM 930 glass filters as well as SRM 935 (stored in a desiccator over magnesium perchlorate) are stable for one to several years.

9.2 *Visible Region*—The absorbance scale in the visible region is tested using glass filters. The wavelengths (nm) of interest and maximum spectral bandwidths (nm) are:

- 440(2.2)
- 465(2.7)
- 590(5.4)
- 635(6.0)

9.2.1 Newer filters also have certified absorbance values at 546.1 nm and data at this wavelength should also be recorded. These filters have certified absorbance values and the precautionary notes stated in the certificate which accompanies this practice should be followed. In cases where verification of the absorbance values of these filters is desired (for example, due to rough handling, improper storage, and age) they should be returned to the NBS for cleaning and recalibration.

9.2.2 Procedure:

9.2.2.1 Examine the glass filters for surface contamination and clean with a soft brush or lens paper if necessary. Measure the temperature of the sample compartment as described in Section 7.

9.2.2.2 Determine the absorbance blank (air versus air) using an appropriate scan speed at the indicated wavelengths. Record these measurements. If large (>0.005A) blank values are observed for recording instruments, adjust the baseline.

Measure the apparent absorbance of each filter at each wavelength and record. Each filter should be oriented in the same manner in the sample holder. If a corrected absorbance reading is outside the acceptable absorbance range, repeat the procedure with a slower scan speed and narrower slit width, if possible, to verify the nonconformity.

9.2.3 Report the visible region calibration data in Table 2.

9.3 *Ultraviolet Region*—The absorbance scale in the ultraviolet region is tested using acidic solutions of potassium dichromate (SRM 935). The wavelengths (nm) of interest and maximum spectral bandwidths (nm) are:

- 235(1.2)
- 257(0.8)
- 313(0.8)
- 345(0.8)
- 350(0.8)

9.3.1 The precautionary notes stated in the certificate that accompanies the standard should be observed.

9.3.2 *Procedure:*

9.3.2.1 Prepare the absorbance standards, solution of potassium dichromate (SRM 935) by transferring 200.0 ± 0.3 , 250.0 ± 0.3 , 300.0 ± 0.3 and 350.0 ± 0.3 mg of SRM 935 to four separate 100 mL volumetric flasks and dilute to volume with distilled water (Absorbance Standard Stock Solutions). Stopper the solutions and mix well. Dilute these solutions by pipetting 20.0 mL of each solution separately to four 1-L volumetric flasks, adding 1 mL of 1M HClO₄ (8.6 mL of 70 % HClO₄/100 mL H₂O) and dilute to volume with distilled water (Absorbance Standard Sample Calibration Solutions). These final calibration solutions contain 40, 50, 60 and 70 mg of SRM 935

per litre of solution, respectively. Prepare a blank solution by diluting 1 mL of 1 M HClO₄ to one L with the same distilled water. Stopper the solutions and mix well.

9.3.2.2 Clean and match the 1-cm solution cells (cuvettes). Measure the temperature of the sample compartment as described in Section 7.

9.3.2.3 Determine the apparent absorbance blank at the indicated wavelengths using solvent in each cuvette. Record these measurements. If large (>0.01A) blank values are observed for recording instruments, adjust the baseline or reclean the cuvettes, or both. Measure the apparent absorbance of each Absorbance Standard Sample Calibration Solution of potassium dichromate in the sample cuvette at each wavelength and record. Rinse the cuvettes several times with the solutions to be measured before they are placed in the sample compartment and maintain the same orientation of the cuvette throughout the procedure. If a corrected apparent absorbance value (^Ameasured) of an Absorbance Standard Sample Calibration is outside the acceptable range, repeat the reading at a slower scan speed and narrower slit width, if possible. If the absorbance readings at all wavelengths for a solution are unacceptable, prepare a fresh solution at the concentration of interest and repeat the absorbance measurements. If non-conformities are verified, corrective service must be performed by a qualified service person. If the user performs this service himself, he should carefully follow the manufacturer's recommended procedure.

9.3.3 Report the ultraviolet region calibration data in Table 3

TABLE 2 UV-Vis Spectrophotometers Absorbance Calibration—Visible Region (NBS SRM__)

Instrument _____
 Date _____
 Temperature _____
 Analyst _____

| Wavelength (nm) and Special Bandwidth (nm) | Filter No. | Calculated Certified Absorbance Range | Absorbance of Filter | Absorbance Blank | ^A Measured ^A | Scan Speed | ΔA (^A Measured— ^B true ^B) | Absorbance Tolerance | |
|--------------------------------------------|------------|---------------------------------------|----------------------|------------------|------------------------------------|------------|----------------------------------------------------------------------|----------------------|------------------|
| | | | | | | | | Conforms | Does Not Conform |
| 440.0 (2.2) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 465.0 (2.7) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 546.1 (6.5) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 590.0 (5.4) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 635.0 (6.0) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |

^A ^A Measured = (Absorbance of Filter—Absorbance Blank).
^B ^B True = mid-value of calculated certified absorbance range.

TABLE 3 UV-Vis Spectrophotometer Absorbance Calibration—Ultraviolet Region (NBS SRM 935)

| Instrument _____ Data _____ Temperature _____ Analyst _____ | Nominal Concentration of Calibration Solution (mg/L) | Actual Concentration | | Apparent Absorbance of Solution | Absorbance Blank | A^{Measured}^A | Scan Speed | ΔA ($A^{\text{measured}} - A^{\text{true}}^B$) | Absorbance Tolerance | |
|----------------------------------------------------------------------|------------------------------------------------------|----------------------------|--------------------------------------------------------|---------------------------------|------------------|-------------------------|------------|----------------------------------------------------------|----------------------|------------------|
| | | Stock Solution (mg/100 mL) | Absorbance Standard Sample Calibration Solution (mg/L) | | | | | | Conforms | Does Not Conform |
| 235 (1.2) | 40 | 0.485–0.499 | | | | | | | | |
| | 50 | 0.607–0.625 | | | | | | | | |
| | 60 | 0.730–0.752 | | | | | | | | |
| | 70 | 0.853–0.879 | | | | | | | | |
| 257 (0.8) | 40 | 0.564–0.582 | | | | | | | | |
| | 50 | 0.706–0.728 | | | | | | | | |
| | 60 | 0.840–0.875 | | | | | | | | |
| | 70 | 0.993–1.023 | | | | | | | | |
| 313 (0.8) | 40 | 0.189–0.195 | | | | | | | | |
| | 50 | 0.237–0.245 | | | | | | | | |
| | 60 | 0.285–0.293 | | | | | | | | |
| | 70 | 0.332–0.342 | | | | | | | | |
| 345 (0.8) | 20 | 0.207–0.216 | | | | | | | | |
| | 40 | 0.419–0.431 | | | | | | | | |
| | 50 | 0.523–0.539 | | | | | | | | |
| | 60 | 0.627–0.647 | | | | | | | | |
| | 70 | 0.733–0.765 | | | | | | | | |
| 350 (0.8) | 20 | 0.210–0.217 | | | | | | | | |
| | 40 | 0.431–0.434 | | | | | | | | |
| | 50 | 0.528–0.543 | | | | | | | | |
| | 60 | 0.633–0.653 | | | | | | | | |
| | 70 | 0.740–0.768 | | | | | | | | |

^A A^{Measured} = (Absorbance of Solution – Absorbance of Blank).

^B A^{True} = mid-value of calculated certified absorbance range.

10. Documentation of Data

10.1 Spectral charts and Tables 1-3 should be retained for reference.

11. Keywords

11.1 molecular spectroscopy; spectrophotometers

 **E 925**

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