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Standard Practice for Monitoring the Calibration of Ultraviolet-Visible Spectrophotometers whose Spectral Bandwidth does not Exceed 2 nm[1]

This standard is issued under the fixed designation E925; 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.

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 reference materials whose properties have been accurately determined. Such materials are readily available, and their use in the tests and measurements described in this practice is satisfactory for evaluating the performance of spectrophotometers whose spectral bandwidth does not exceed the value for which the intrinsic or certified properties are valid. A compromise maximum permissible spectral bandwidth of 2 nm is recommended for the reference materials and error tolerances recommended here.

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 ultraviolet (UV) and visible regions is determined using the sharp absorption bands of a holmium oxide glass or solution filter. The absorbance scale accuracy in the UV region (235 to 350 nm) is determined using acidic solutions of potassium dichromate. In the visible region (440 to 635 nm) the absorbance accuracy is determined using individually certified neutral density glass filters. The use of these reference materials provides a valid and relatively simple means to test the errors in the wavelength and absorbance scales of small spectral bandwidth spectrophotometers in the spectral ranges indicated. A simplified version of the opaque filter method is provided as a test for excessive stray radiant energy.

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[2] within the wavelength range of 200 to 700 nm and the absorbance range of 0 to 2. 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 instruments for which the spectral bandwidth does not exceed 2 nm and for which the manufacturer's specifications for wavelength and absorbance accuracy do not exceed the performance tolerances employed here. This practice employs an illustrative tolerance of ±1 % relative for the error of the absorbance scale over the range of 0.2 to 2.0, and of ±1.0 nm for the error of the wavelength scale. A suggested maximum stray radiant power ratio of 4×10^{-4} yields <1 % absorbance bias at an absorbance of 2. These tolerances are chosen to be compatible with many chemical applications while comfortably exceeding the uncertainty of the certified values for the reference materials and typical manufacturer's specifications for error in the wavelength and absorbance scales of the instrument under test. The user is encouraged to develop and use tolerance values more appropriate to the requirements of the end use application. This procedure is designed to verify quantitative performance on an ongoing

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[1] This practice is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and Separation Science and is the direct responsibility of Subcommittee E13.01 on Ultra-Violet, Visible, and Luminescence Spectroscopy.

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

basis and to compare one instrument's performance with that of other similar units. Refer to Practice E275 to extensively evaluate the performance of an instrument.

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 tested 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 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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:*[3]

E131 Terminology Relating to Molecular Spectroscopy
E169 Practices for General Techniques of Ultraviolet-Visible 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
E1866 Guide for Establishing Spectrophotometer Performance Tests

2.2 *NIST Publications:*[4]

NIST Special Publication 260-54 Certification and Use of Acidic Potassium Dichromate Solutions As An Ultraviolet Absorbance Standard
NIST Special Publication 260-102 Holmium Oxide Solution Wavelength Standard from 240 to 640 nm—SRM 2034
NIST Special Publication 260-116 Glass Filters as a Standard Reference Material for Spectrophotometry—Selection, Preparation, Certification, and Use of SRM 930 and SRM 1930
NIST Special Publication 260-140 Technical Specifications for Certification of Spectrophotometric NTRMs

2.3 *ISO Publications:*[5]

ISO 17025 General Requirements for the Competence of Testing and Calibration Laboratories
ISO Guide 34 General Requirements for the Competence of Reference Material Producers

3. Terminology

3.1 *Definitions:*

3.1.1 For the definitions of terms used in this practice, refer to Terminology E131.

3.1.2 For a description of the instrumental parameters evaluated in this practice, refer to Practice E275.

3.1.3 For a description of quantitative ultraviolet spectrophotometric techniques, refer to Practice E169.

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 monitoring 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-Monitoring Procedure

5.1 Reference to this practice in any spectrophotometric calibration-monitoring 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 data obtained are within the stated tolerances from 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 reference materials, all the components of the spectrophotometer must be functioning properly. In addition, the temperature of the specimen compartment should be between 20 and 25°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 (**1, 2**)[6] or solution standard (NIST Special Publication 260-102) may be used for evaluating wavelength accuracy. The glass and solution standards are both available commercially from reference material producers, in the sealed cuvette format (a cuvette-shaped metal holder is used for the glass) or as a bottled solution, prepared from high purity Holmium Oxide (> 99.99 %), where value assignment is by self assertion (Note 1).

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[3] 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.

[4] Available from National Technical Information Service (NTIS), 5301 Shawnee Rd., Alexandria, VA 22312, http://www.ntis.gov.

[5] Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, http://www.iso.org.

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

A purchaser should require certification by the supplier that the wavelengths of the absorption bands are within 0.2-nm of the values given in Ref. (**2**), and reported below. The appropriate solution standard is 4 % (mass fraction) holmium oxide in 10 % (volume fraction) perchloric acid, contained in a 10-mm path length cuvette. For this material, the transmittance minima of 18 absorption bands have been certified by a multi-laboratory inter-comparison, at the highest level, allowing the peak value assignments as an intrinsic wavelength standard (**3**). Absorbance maxima or transmittance minima must be located within ±1 nm of the wavelengths given below:

Glass Filter[A]	Dilute Acidic Solution[B]
241.5 nm[C]	241.1 nm
. . .	249.9 nm
279.3 nm	278.1 nm
287.6 nm	287.2 nm
333.8 nm	333.5 nm
. . .	345.4 nm
360.8 nm	361.3 nm
385.8 nm	385.6 nm
418.5 nm	416.3 nm
453.4 nm	. . . [D]
459.9 nm	467.8 nm
. . .	485.3 nm
536.4 nm	536.6 nm
637.5 nm	640.5 nm

[A] Wavelengths taken from Ref. (**2**) for Corning Glass Works Code 3130 glass, superceded by Corning Glass Works Code 3131 glass and Kopp Glass Code 3131 glass, for which the wavelengths are also valid.

[B] Wavelengths rounded to 0.1 nm for a 1-nm spectral bandwidth taken from Ref. (**3**).

[C] May not be usable, depending on the base glass of the filter.

[D] Peak omitted because it resolves into a doublet at spectral bandwidth values less than 1 nm.

NOTE 1—'Self assertion' may take the form of value assignment and certification in many forms. Some specific examples are:

(*1*) By a national metrology institute (NMI),

(*2*) By an ISO 17025 and ISO Guide 34 accredited Reference Material producer, and

(*3*) By a laboratory claiming 'traceability' to an NMI.

In all cases, the user should be satisfied that the quality of the value assignment data meets the laboratory requirements.

7.1.1 If the observed absorption bands of the holmium oxide glass or solution deviate by more than ±1 nm from the values stated, then corrective service must by performed on the instrument by qualified personnel. If the user performs this service, the manufacturer's recommended procedure should be followed carefully.

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

7.1.3 Computer based peak location algorithms that may be used to assign absorbance maxima or transmittance minima are discussed in 7.6 of Guide E1866. It should be noted that peak asymmetries in the holmium oxide reference materials are such that digital filter widths should be smaller than the full-width-half-maximum recommendation of that guide.

7.1.4 In the absence of drift or slippage in the wavelength drive train, repeatability of the band positions should be on the order of ±0.1 nm for a given instrument, especially with the use of a computer based peak location algorithm.

7.2 *Procedure:*

7.2.1 Examine the holmium oxide reference material and remove any surface contamination using a soft brush or lint-free cloth. Measure the temperature of the sample compartment by placing an appropriate sensor into the cell compartment of a stabilized instrument and replacing the compartment cover securely. Place the sensor as close as possible to the actual position that will be occupied by the standard. After a suitable period of time record the temperature reading, remove the sensor, and resume normal operations.

7.2.2 Record the blank absorbance or transmittance (air versus air) spectrum at the desired resolution and at the appropriate wavelength intervals and scan speeds, in order to perform any necessary baseline adjustments. The wavelength intervals should be no greater than the spectral bandwidth used. Acquire the appropriate spectrum of the holmium oxide reference material with respect to air and baseline correct if necessary using the blank spectrum. Record the wavelengths of the positions of the relevant bands, and compare these values to the expected values. If large discrepancies (>1 nm) exist between the true and measured wavelengths, repeat the procedure at a slower scan speed and smaller spectral bandwidth, if possible, to verify the nonconformity.

7.2.3 Report the wavelength calibration data in the manner of Table 1, given as an example for the holmium oxide glass reference material.

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 or NaL, 10.0 g/L in H_2O	220 nm
$NaNo_2$, 50.0 g/L in H_2O	370 nm

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

Instrument _________
Date ______________
Temperature ________
Analyst ____________

Wavelength Calibration: Holmium Oxide Filter

True Wavelength (nm)	Observed Wavelength (nm)	Difference (nm)	Conformance	
			Does	Does Not
241.5 ± 1				
279.3 ± 1				
287.6 ± 1				
333.8 ± 1				
360.8 ± 1				
385.8 ± 1				
418.5 ± 1				
453.4 ± 1				
459.9 ± 1				
536.4 ± 1				
637.5 ± 1				

Stray Radiant Power Ratio

Wavelength (nm)	Transmittance or Absorbance	Conforms	Does Not Conform
220			
340			

8.1.1 Reagent grade materials should be used for these solutions. They are essentially opaque at the indicated wavelengths; any observed transmittance is equivalent to the effective SRPR.

8.1.2 An acceptable level of SRPR depends on the spectral character and absorbance level of the sample under investigation. However, an upper limit of 4×10^{-4} is consistent with a worst-case absorbance bias of ~1 % at the upper limit of the absorbance range ($0 < A \leq 2$) covered by this practice, and is suggested in the absence of other criteria.

8.1.3 While the stray radiant power ratio is equivalent to the transmittance described previously, it is often more convenient to make the measurement in the absorbance mode and mathematically convert absorbance to transmittance. The value quoted in 8.1.2 (4×10^{-4}) equates to an absorbance value of 3.4*A*.

8.1.4 An excessive SRPR usually arises from dust, scratches, or corrosion on the collimator or disperser, or both. Qualified personnel should correct this problem. 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.

8.2 *Procedure:*

8.2.1 Use the visible light source lamp in the 340 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 Refer to Test Method E387 if the dynamic range of the readout electronics of the instrument is not adequate for the direct measurement of SRPR as described here.

8.2.4 In the manner of Table 1, report the transmittance or absorbance of these solutions. Note whether the effective stray radiant power ratio exceeds the suggested tolerance of 4×10^{-4} or the user-defined tolerance.

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 reference materials with known absorbances. The absorbance scale accuracy in the ultraviolet region (235 to 350 nm) is determined using acidic solutions of potassium dichromate as described in NIST Special Publication 260-54. In the visible region (440 to 635 nm) the absorbance accuracy is determined using certified neutral density glass filters as described in NIST Special Publication 260-116 and NIST Special Publication 260-140. The certified absorbances should be traceable to the regular transmittance scale maintained by an NMI.

9.1.1 If the blank-corrected absorbances (A_{corr}) of the standards are outside the acceptable range, then corrective service must be performed on the instrument by qualified personnel. If the user performs this service, the manufacturer's recommended procedure should be followed carefully.

9.1.2 An acceptable absorbance range for each standard for any instrument must be determined based on the instrument manufacturer's specifications and on the analytical demands of the end-use application of the instrument. As a guide to the acceptability of photometric accuracy data a tolerance of ±1.0 % relative ($0.2 \leq A \leq 2.0$) is employed in this practice. The user is encouraged to establish tolerance limits more appropriate to the application in question, and use the tables of this practice as templates for custom tables that reflect the appropriate tolerances. One approach often used in defining these limits is to linearly add the certified expanded uncertainty budget ($k = 2$) for a given reference material, to the manufacturer quoted instrument photometric accuracy specification.

9.1.3 Rigorous treatment of the construction and use of an absorbance correction curve for high accuracy work is beyond the scope of this practice.

9.1.4 Studies by NIST and other ISO 17025 and ISO Guide 34 accredited organizations have indicated that solutions of acidic potassium dichromate are stable for at least six months when prepared in the manner described in 9.3.2.1 and stored in the dark in well-stoppered 1-L volumetric flasks, and for at least two years when permanently sealed in ampoules or far UV quartz cuvettes, by heat fusion. Neutral density glass filters are certified by different sources for periods of from two to five years, with appropriately adjusted uncertainties.

9.2 *Visible Region*—The absorbance scale in the visible region is tested using filters of a proprietary neutral glass. The construction and certification of such filters is described in some detail in NIST Special Publication 260-116 and NIST Special Publication . Traceability of the certified absorbance values to the transmittance scale maintained by the Analytical Chemistry Division (ACD) of NIST is supported by NIST for commercial participants in the NIST Traceable Reference Materials (NTRM) program, or by self assertion (Note 1) for other commercial sources. Traceability of these filters is normally maintained through NIST SRM 930 filters with nominal absorbances of 0.5, 0.7, and 1.0 and SRM 1930 filters with absorbances of 0.3, 1.5, and 2.0. (A letter series designation for SRM 930 is periodically adjusted without significant effect for this practice.) The wavelengths for which certified absorbance values are reported for individual filters are close to local extrema in the nearly-neutral glass to minimize the effect of wavelength error on the measured transmittance:

440.0 nm
465.0 nm
546.1 nm
590.0 nm
635.0 nm

Neutral density glass filters are also available from the National Physical Laboratory (NPL) of the UK and from commercial sources asserting traceability to the regular transmittance scale maintained by NPL.

9.2.1 These filters have individually certified absorbance values and the precautionary notes stated in the certificate that accompanies the filters should be followed. In cases where recertification of the absorbance values of these filters is required (due to expiration or improper storage or handling) they should be returned to the certifying laboratory for cleaning and recertification.

9.2.2 *Procedure:*

9.2.2.1 Examine the glass filters for surface contamination and clean with a bulb-type of air puffer if necessary. Any other attempt to clean the filters invalidates the certification. Measure the temperature of the sample compartment as described in Section 7.

9.2.2.2 Determine the absorbance blank (air versus air absorbance value) at the indicated wavelengths. Record these measurements. If large (>0.001*A*) blank values are observed, use these to blank-correct measured apparent absorbances by subtraction. Measure the apparent absorbance of each filter at each wavelength versus air. 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 longer integration time and smaller spectral bandwidth, if possible, to verify the nonconformity.

9.2.3 Report the visible region validation data in the manner of Table 2, constructed for a set of three filters of the nominal absorbances of NIST SRM 930.

9.3 *Ultraviolet Region*—The absorbance scale in the ultraviolet region is tested using acidic solutions of potassium dichromate (available from NIST as SRM 935a). The wavelengths of interest are:

235 nm
257 nm
313 nm
350 nm

NOTE 2—Acidic potassium dichromate solutions specifically prepared for spectrophotometric validation are also available commercially in solution, sealed ampoules, and sealed cuvette formats. Portions of the procedure below, for the powder form, will not be required for these forms. Certified values and expiration dates that accompany such preparations should be observed.

9.3.1 The precautionary notes stated in the certificate and the material safety data sheet (MSDS) for SRM 935a should be observed. These documents are available from the NIST internet site at www.nist.gov under the Standard Reference Materials Program online catalog.

9.3.2 *Procedure:*

9.3.2.1 Prepare the absorbance standard solutions of potassium dichromate by transferring 200.0 ± 0.3, 300.0 ± 0.3, 400.0 ± 0.3, and 500.0 ± 0.3 mg of the powder 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 1*M* $HClO_4$ (8.6 mL of 70 % $HClO_4$/100 mL H_2O) and diluting to volume with distilled water (Absorbance Standard Sample Calibration Solutions). These final calibration solutions contain 40, 60, 80, and 100 mg of potassium dichromate per litre of solution, respectively. Prepare a blank solution by diluting 1 mL of 1 *M* $HClO_4$ 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.

TABLE 2 UV-VIS Spectrophotometers Absorbance Calibration—Visible Region

Instrument __________
Date ______________
Temperature _______
Analyst ___________

Wavelength (nm)	Filter No.	A_{nom}[A]	A_{cert}[A]	A_{corr}[A]	Bias[B]	Tolerance[C]	Conformance[D]	
							Does	Does Not
440.0		0.5				0.005		
		0.7				0.007		
		1.0				0.010		
465.0		0.5				0.005		
		0.7				0.007		
		1.0				0.010		
546.1		0.5				0.005		
		0.7				0.007		
		1.0				0.010		
590.0		0.5				0.005		
		0.7				0.007		
		1.0				0.010		
635.0		0.5				0.005		
		0.7				0.007		
		1.0				0.010		

[A] A_{nom} = nominal absorbance; A_{cert} = certified absorbance; A_{corr} = measured absorbance, blank corrected as necessary.

[B] Bias = $A_{corr} - A_{cert}$.

[C] Tolerance taken for example as 1 % of the nominal. User to assign as appropriate for each application.

[D] Measurement conforms for |Bias| ≤ Tolerance; measurement does not conform for |Bias| > Tolerance.

TABLE 3 UV-VIS Spectrophotometer Absorbance Calibration—Ultraviolet Region (Potassium Dichromate)

Instrument _________
Date ______________
Temperature _______
Analyst ___________

Wavelength (nm)	Solution (mg/L)	A_{cert}[A]	A_{meas}[B]	A_{blank}[B]	A_{corr}[B]	Bias[C]	Tolerance[D]	Conformance[E]	
								Does	Does Not
235.0	40	0.492					0.005		
	60	0.741					0.007		
	80	0.991					0.010		
	100	1.243					0.012		
257.0	40	0.573					0.006		
	60	0.862					0.009		
	80	1.154					0.012		
	100	1.449					0.014		
313.0	40	0.192					0.002		
	60	0.289					0.003		
	80	0.386					0.004		
	100	0.483					0.005		
350.0	40	0.427					0.004		
	60	0.645					0.006		
	80	0.860					0.009		
	100	1.071					0.011		

[A] A_{cert} = certified absorbance, computed from SRM 935a certified specific absorbance values for the given solution and a 10-mm pathlength.

[B] A_{meas} = measured solution absorbance; A_{blank} = measured blank absorbance; $A_{corr} = A_{meas} - A_{blank}$.

[C] Bias = $A_{corr} - A_{cert}$.

[D] Tolerance computed for illustrative purposes as 1 % of the certified absorbance. User may substitute appropriate tolerances.

[E] Corrected absorbance conforms when $|\text{Bias}| \leq$ Tolerance.

9.3.2.3 Determine the apparent absorbance blank at the indicated wavelengths using solvent in each cuvette. Record these measurements. If large (>0.01*A*) blank values are observed, re-cleaning the cuvettes may be necessary. 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 a cuvette throughout the procedure. If a corrected apparent absorbance value (A_{corr}) of an Absorbance Standard Sample Calibration is outside the acceptable range, repeat the reading with a longer integration time and smaller spectral bandwidth, 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 qualified personnel. If the user performs this service, the manufacturer's recommended procedure should be followed carefully.

9.3.3 Report the ultraviolet region calibration data in the manner of Table 3.

10. Documentation of Data

10.1 Spectral charts and tables should be retained for reference.

11. Keywords

11.1 absorbance; molecular spectroscopy; reference materials; spectrophotometers; UV/visible; wavelength

REFERENCES

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