Designation: E 1657 – 98

Standard Practice for Testing Variable-Wavelength Photometric Detectors Used in Liquid Chromatography[1]

This standard is issued under the fixed designation E 1657; 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 This practice is intended to serve as a guide for the testing of the performance of a variable-wavelength photometric detector (VWPD) used as the detection component of a liquid-chromatographic (LC) system operating at one or more wavelengths in the range 190 to 800 nm. Many of the measurements are made at 254 nm for consistency with Practice E 685. Measurements at other wavelengths are optional.

1.2 This practice is intended to describe the performance of the detector both independently of the chromatographic system (static conditions) and with flowing solvent (dynamic conditions).

1.3 For general liquid chromatographic procedures, consult Refs **(1-9)**.[2]

1.4 For general information concerning the principles, construction, operation, and evaluation of liquid-chromatography detectors, see Refs **(10, 11)** in addition to the sections devoted to detectors in Refs **(1-7)**.

1.5 The values stated in SI units are to be regarded as standard.

1.6 *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 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers[3]

E 682 Practice for Liquid Chromatography Terms and Relationships[4]

E 685 Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography[4]

3. Terminology

3.1 *Definitions:*

3.1.1 *absorbance calibration*—the procedure that verifies that the absorbance scale is correct within ±5 %.

3.1.2 *drift*—the average slope of the noise envelope expressed in absorbance units per hour (AU/h) as measured over a period of 1 h.

3.1.3 *dynamic*—under conditions of a flow rate of 1.0 mL/min.

3.1.4 *linear range—of a VWPD*, the range of concentrations of a test substance in a test solvent over which the ratio of response of the detector versus concentration of test substance is constant to within 5 % as determined from the linearity plot specified in 7.1.2 and illustrated in Fig. 1. The *linear range* should be expressed as the ratio of the upper limit of linearity obtained from the plot to either a) the lower linear concentration, or b) the *minimum detectable* concentration, if the *minimum detectable* concentration is greater than the lower linear concentration.

3.1.5 *long-term noise*—the maximum amplitude in AU for all random variations of the detector signal of frequencies between 6 and 60 cycles per hour (0.1 and 1.0 cycles per min).

3.1.5.1 *Discussion*—It represents noise that can be mistaken for a late-eluting peak. This noise corresponds to the observed noise only and may not always be present.

3.1.6 *minimum detectability— of a VWPD*, that concentration of a specific solute in a specific solvent that results in a detector response corresponding to twice the static short-term noise.

3.1.6.1 *Discussion*—The static short-term noise is a measurement of peak-to-peak noise. A statistical approach to noise suggests that a value of three times the rms (root-mean-square) noise would insure that any value outside this range would not be noise with a confidence level of greater than 99 %. Since peak-to-peak noise is approximately five times the rms noise **(12)**, the minimum detectability defined in this practice is a more conservative estimate.

3.1.7 *response time (speed of output)— the detector*, the time required for the detector output to change from 10 % to 90 % of the new equilibrium value when the composition of the mobile phase is changed in a stepwise manner, within the linear range of the detector.

3.1.7.1 *Discussion*—Because the detector volume is very small and the transport rate is not diffusion dependent, the

[1] This practice is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.19 on Chromatography.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this practice.

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

[4] *Annual Book of ASTM Standards*, Vol 14.01.

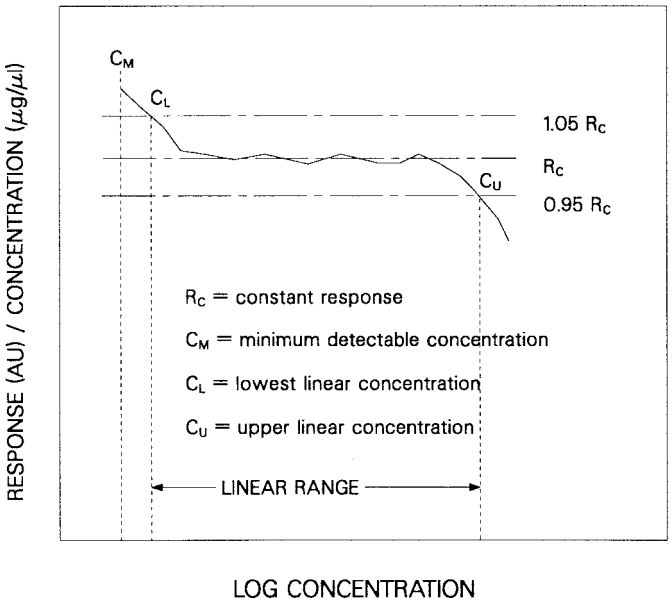

FIG. 1 Example of Linearity Plot for a Variable-Wavelength Detector

response time is generally fast enough to be unimportant. It is generally comparable to the response time of the recorder and dependent on the response time of the detector electrometer and on the recorder amplifier. Factors that affect the observed response time include the true detector response time, electronic filtering, and system band-broadening.

3.1.8 *short-term noise*—the maximum amplitude, peak to peak, in AU for all random variations of the detector signal of a frequency greater than one cycle per minute.

3.1.8.1 *Discussion*—It determines the smallest signal detectable by a VWPD, limits the precision attainable in quantitation of trace-level samples, and sets the lower limit on linearity. This noise corresponds to the observed noise only.

3.1.9 *static*—under conditions of no flow.

3.1.10 *wavelength accuracy*—the deviation of the observed wavelength maximum from the maximum of a known test substance.

3.1.11 *wavelength precision*—a measure of the ability of a VWPD to return to the same spectral position as measured by the reproducibility of absorbance values when the detector is reset to a wavelength maximum of a known test substance.

4. Significance and Use

4.1 Although it is possible to observe and measure each of the several characteristics of a detector under different and unique conditions, it is the intent of this practice that a complete set of detector specifications should be obtained *under the same operating conditions.* It should also be noted that to completely specify a detector's capability, its performance should be measured at several sets of conditions within the useful range of the detector. The terms and tests described in this practice are sufficiently general that they may be used regardless of the ultimate operating parameters.

4.2 Linearity and response time of the recorder or other readout device used should be such that they do not distort or otherwise interfere with the performance of the detector. This requires adjusting the gain, damping, and calibration in accordance with the manufacturer's directions. If additional electronic filters or amplifiers are used between the detector and the final readout device, their characteristics should also first be established.

5. Noise and Drift

5.1 *Test Conditions*—Pure, degassed methanol[5] shall be used in the sample cell. Air or nitrogen shall be used in the reference cell if there is one. Nitrogen is preferred where the presence of high-voltage equipment makes it likely that there is ozone in the air. Protect the entire system from temperature fluctuations because these will lead to detectable drift.

5.1.1 The detector should be located at the test site and turned on at least 24 h before the start of testing. Insufficient warm-up may result in drift in excess of the actual value for the detector. The detector wavelength should be set to 254 nm.

5.2 *Methods of Measurement*:

5.2.1 Connect a suitable device (see Note 1) between the pump and the detector to provide at least 75 kPa (500 psi) back pressure at 1.0 mL/min flow of methanol. Connect a short length (about 100 mm) of 0.25-mm (0.01-in.) internal-diameter stainless steel tubing to the outlet tube of the detector to retard bubble formation. Connect the recorder to the proper detector output channels.

NOTE 1—Suggested devices include (*a*) 2 to 4 m of 0.1-mm (0.004-in.) internal-diameter stainless steel tubing, (*b*) about 250 mm of 0.25 to 0.5 mm (0.01 to 0.02-in.) internal-diameter stainless steel tubing crimped with pliers or cutters, or (*c*) a constant back-pressure valve located between the pump and the injector.

5.2.2 Repeatedly rinse the reservoir and chromatographic system, including the detector, with degassed methanol to remove from the system all other solvents, any soluble material, and any entrained gasses. Fill the reservoir with methanol and pump this solvent through the system for at least 30 min to complete the system cleanup.

5.2.3 Air or nitrogen is used in the reference cell, if any. Ensure that the cell is clean, free of dust, and completely dry.

5.2.4 To perform the static test, cease pumping and allow the chromatographic system to stabilize for at least 1 h at room temperature without flow. Set the attenuator at maximum sensitivity (lowest attenuation), that is, the setting for the smallest value of absorbance units full-scale (AUFS). Adjust the response time as close as possible to 2 s for a VWPD that has a variable response time (see Note 2). Record the response time used. Adjust the detector output to near midscale on the readout device. Record at least 1 h of detector signal under these conditions, during which time the ambient temperature should not change by more than 2°C.

NOTE 2—Time constant is converted to response time by multiplying by the factor 2.2. The effect of electronic filtering on observed noise may be studied by repeating the noise measurements for a series of response-time settings.

5.2.5 Draw pairs of parallel lines, each pair corresponding to between 0.5 and 1 min in length, to form an envelope of *all*

[5] Distilled-in-glass or liquid-chromatography grade. Complete freedom from particles may require filtration, for example, through a 0.45-μm membrane filter.

observed random variations over any 15-min period (see Fig. 2). Draw the parallel lines in such a way as to minimize the distance between them. Measure the vertical distance, in AU, between the lines. Calculate the average value over all the segments. Divide this value by the cell length in centimeters to obtain the *static short-term noise*.

5.2.6 Now mark the center of each segment over the 15-min period of the static short-term noise measurement. Draw a series of parallel lines encompassing these centers, each pair corresponding to 10 min in length, and choose that pair of lines whose vertical distance apart is greatest (see Fig. 2). Divide this distance in AU by the cell length in centimeters to obtain the *static long-term noise*.

5.2.7 Draw the pair of parallel lines that minimizes the vertical distance separating these lines over the 1 h of measurement (Fig. 2). The slope of either line is the *static drift* expressed in AU/h.

5.2.8 Set the pump to deliver 1.0 mL/min under the same conditions of tubing, solvent, and temperature as in 5.2.1-5.2.3. Allow 15 min for the system to stabilize. Record at least 1 h of signal under these flowing conditions, during which time the ambient temperature should not change by more than 2°C.

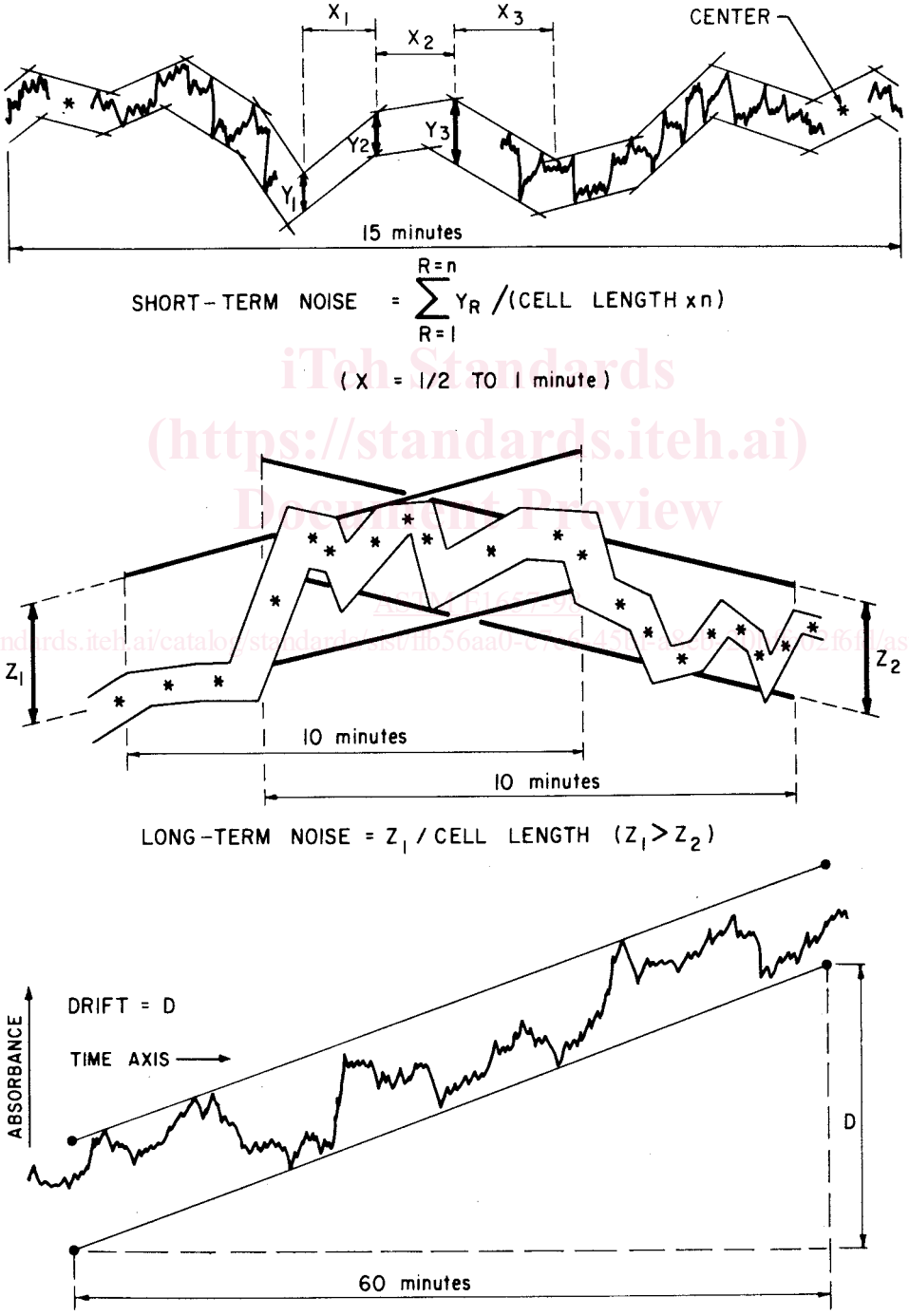

FIG. 2 Example for the Measurement of the Noise and Drift of a VWD (Chart Recorder Output)

5.2.9 Draw pairs of parallel lines, measure the vertical distances, and calculate the *dynamic short-term noise* following the procedure of 5.2.5.

5.2.10 Make the measurement for the *dynamic long-term noise* following the procedure outlined in 5.2.6.

5.2.11 Draw the pair of parallel lines as directed in 5.2.7. The slope of these lines is the *dynamic drift*.

5.2.12 The actual noise of the system may be larger or smaller than the observed values, depending upon the method of data collection, or signal monitoring of the detector, since observed noise is a function of the frequency, speed of response, and bandwidth of the readout device.

6. Wavelength Accuracy and Precision

6.1 The *wavelength accuracy and precision of a VWPD* are important parameters for the performance of chromatographic methods. The wavelength specified in the method may be critical to the detection of different compounds having different absorption spectra. The stated *linear range* of the method may be compromised if the wavelength is inaccurate. Further, the precision of adjusting the detector to the same wavelength should also be known. The wavelength of a VWPD is determined by the monochromator and the optical alignment of the detector. The optical alignment is performed by the manufacturer and usually does not need readjustment. Some detectors require alignment of the lamp after replacement. This procedure verifies that the detector is properly aligned and meets the manufacturer's specifications for *wavelength accuracy and precision.*

6.2 *Method of Measurement—Wavelength Accuracy*—For the determination of the *wavelength accuracy of a VWPD*, **(13)** a solution of a compound with known absorbance maxima is introduced into the cell. The measured maxima are compared to the known maxima for the compound. There are several acceptable compounds and solvents.[6] The following procedure is recommended (Note 3).

NOTE 3—The recommended procedure is covered under U.S. Patent 4,836,673. The American Society for Testing and Materials takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility. Alternative procedures will be considered.

6.2.1 Prepare the test solution. For example, dissolve 2 g of erbium perchlorate hexahydrate[7] in 25 mL water.[8] The nominal concentration is 0.14 M. Filter the solution with an appropriate filter[9] to ensure the sample is free of particles.

NOTE 4—This can be conveniently done by addng water to a 2 g vial of erbium perchlorate hexahydrate to dissolve the solid. Transfer the contents to a 25 mL volumetric flask and make up to volume with water. While reasonable care should be observed in transferring the dissolved erbium perchlorate into the volumetric flask, the final solution is not used quantitatively.

6.2.2 Turn on the detector and allow it to warm up according to the manufacturer's recommendations. Thoroughly flush the detector cell with water preferably from the same source as that to make up the test solution. (If using another test compound, be sure to use the same solvent as the test solution.) Set the detector wavelength to 250 nm. Zero the absorbance of the detector. (Some detectors will automatically zero the detector after changing wavelengths.) Flush the cell with at least 1 mL of the erbium test solution. Record the absorbance reading. Increase the wavelength by 1 nm. Flush the cell with at least 1 mL of water. Zero the absorbance of the detector. Flush the cell with the erbium test solution and record the absorbance. Repeat the procedure in 0.5 to 1.0 nm increments until reaching 260 nm.

6.2.3 Plot absorbance versus wavelength and determine the maximum absorbance. (See Fig. 3) Compare the calculated maximum to the maximum for erbium perchlorate of 255 nm (see Note 4). Report the nominal and calculated maximum of the test sample. The calculated maximum should be within the manufacturer's specification for *wavelength accuracy.* If the detector does not meet specifications, service on the detector to realign the lamp or the monochromator, or both, is indicated.

NOTE 5—Since VWPD detectors can have a large bandpass, it is not necessary to determine the wavelength accuracy to the same degree as that expected for spectrophotometers. The known wavelengths have been reported to the nearest whole nanometer.

6.2.4 The test may be repeated at a second wavelength such as 379 nm or 522 nm. The test at 522 nm would be critical if a second light source is used for detection in the visible range.

6.3 *Method of Measurement—Wavelength Precision*—For determination of the *wavelength precision of a VWPD*, the absorbance of a solution of a compound at the known wavelength maximum of the compound is measured repeated after the wavelength is reset by the operator. This procedure tests the

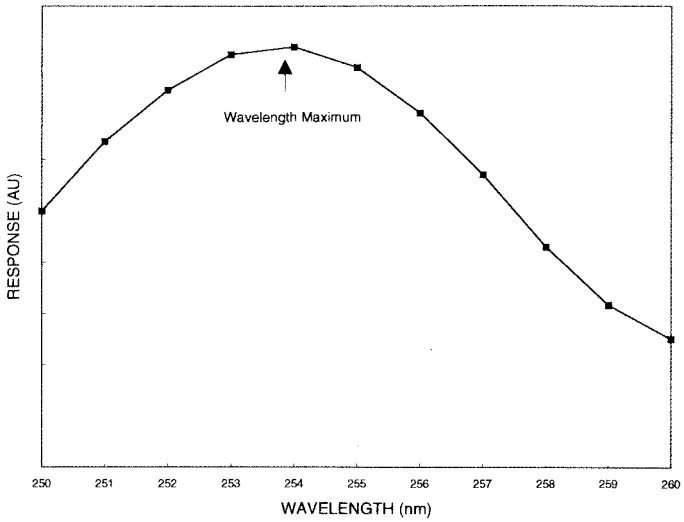

FIG. 3 Example of Wavelength Accuracy Test Plot

[6] In addition to erbium perchlorate, holium perchlorate in water (241 nm, 362 nm, 536–537 nm), and naphthalene in methanol (218 nm) are also possible standards.

[7] Erbium perchlorate hexahydrate can be purchased in 2 g vials from Alfa Research Chemicals. Perchlorates are strong oxidizing agents. Observe all precautions on the Material Safety Data Sheet. The test solution is stable for several weeks as a wavelength standard. However, since detector evaluation is normally infrequent, it is recommended that the solutions be prepared shortly before use and discarded after use. Use approval disposal procedures.

[8] Water, liquid chromatographic grade or equivalent.

[9] For example, a 0.45µ filter suitable for aqueous filtration.