



Designation: E1657 – 98 (Reapproved 2019)

Standard Practice for Testing Variable-Wavelength Photometric Detectors Used in Liquid Chromatography¹

This standard is issued under the fixed designation E1657; 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 covers 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 E685. 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. No other units of measurement are included in this 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

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

Current edition approved Dec. 1, 2019. Published December 2019. Originally approved in 1994. Last previous edition approved in 2011 as E1657-98 (2011). DOI: 10.1520/E1657-98R19.

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

2. Referenced Documents

2.1 *ASTM Standard*.³

E685 Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography

3. Terminology

3.1 *Definitions*:

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

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 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.

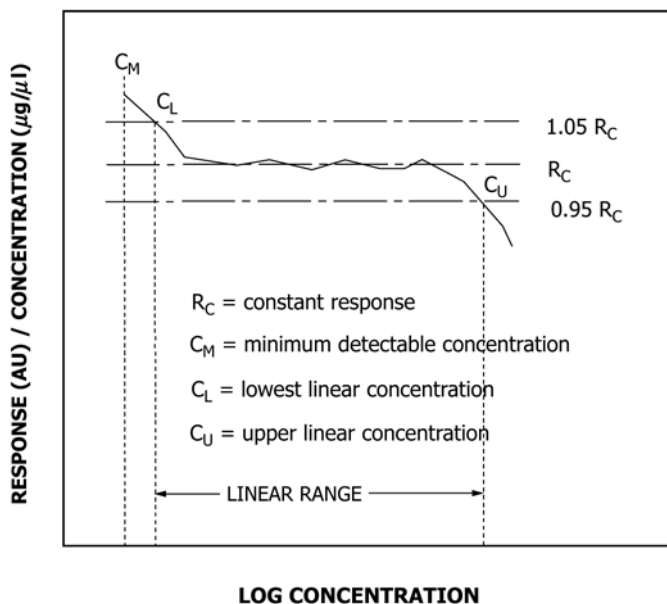


FIG. 1 Example of Linearity Plot for a Variable-Wavelength Detector

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 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 methanol⁴ 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.

⁴ Distilled-in-glass or liquid-chromatography grade. Complete freedom from particles may require filtration, for example, through a 0.45 μm membrane filter.

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 observed random variations over any 15 min period (see Fig.

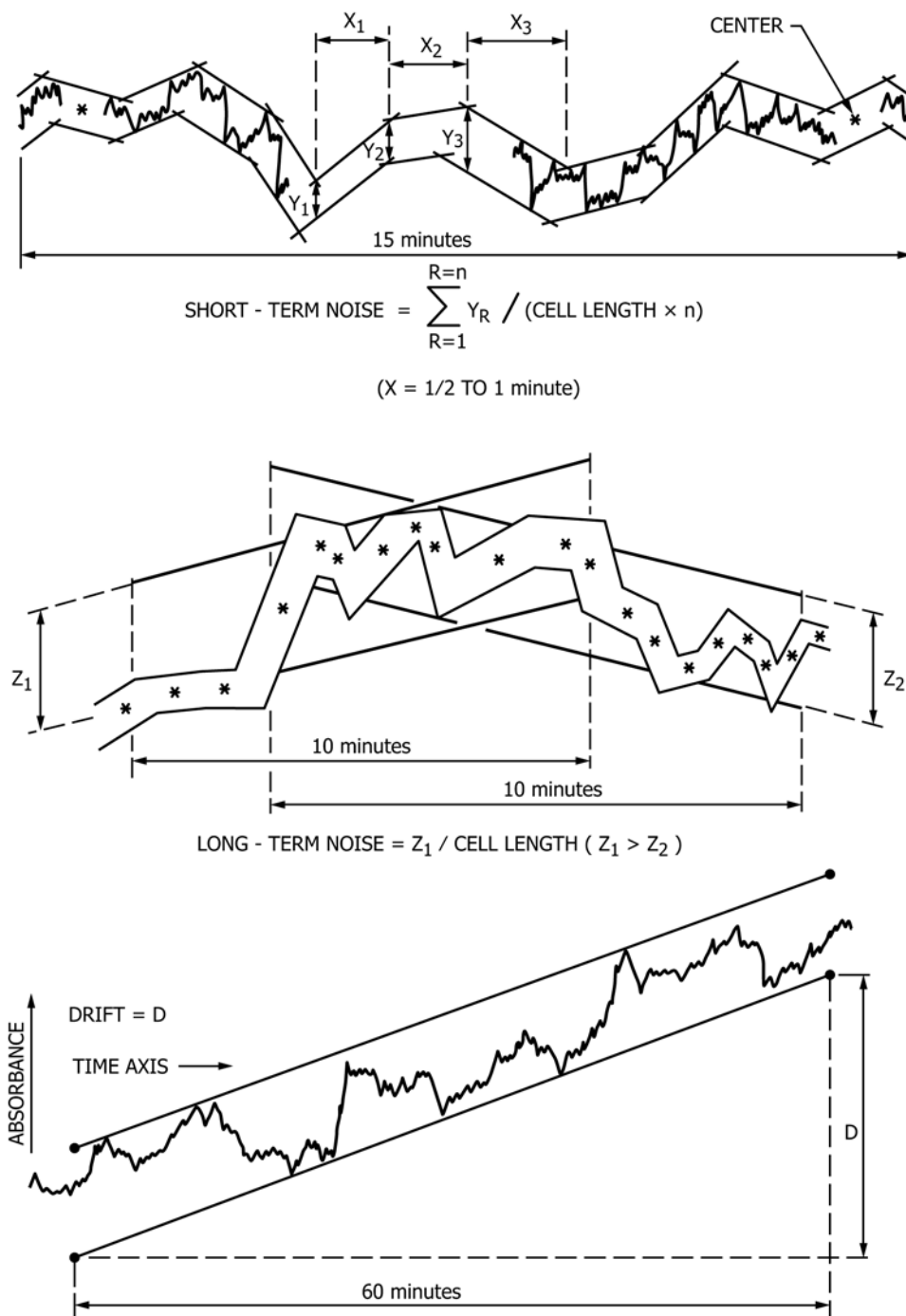


FIG. 2 Example for the Measurement of the Noise and Drift of a VWPD (Chart Recorder Output)