



# Standard Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography<sup>1</sup>

This standard is issued under the fixed designation E 685; 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 ( $\epsilon$ ) 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 photometric detector (PD) used as the detection component of a liquid-chromatographic (LC) system operating at one or more fixed wavelengths in the range 210 to 800 nm. Measurements are made at 254 nm, if possible, and are optional at other wavelengths.

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).<sup>2</sup>

1.4 For general information concerning the principles, construction, operation, and evaluation of liquid-chromatography detectors, see Refs (10 and 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 problems, 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<sup>3</sup>

E 682 Practice for Liquid Chromatography Terms and Relationships<sup>4</sup>

## 3. Terminology

### 3.1 Definitions:

<sup>1</sup> 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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<sup>2</sup> The boldface numbers in parentheses refer to the list of references at the end of this practice.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 14.02.

<sup>4</sup> *Annual Book of ASTM Standards*, Vol 03.06.

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 PD*, the range of concentrations of a test substance in a mobile phase over which the response of the detector is constant to within 5% as determined from the linearity plot specified below and illustrated in Fig. 1. The linear range should be expressed as the ratio of the highest concentration to the minimum detectable concentration or the lowest linear concentration, whichever is greatest.

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 PD*, that concentration of a specific solute in a specific solvent that results in a detector response corresponding to twice the static short-term noise.

3.1.7 *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 PD, 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.

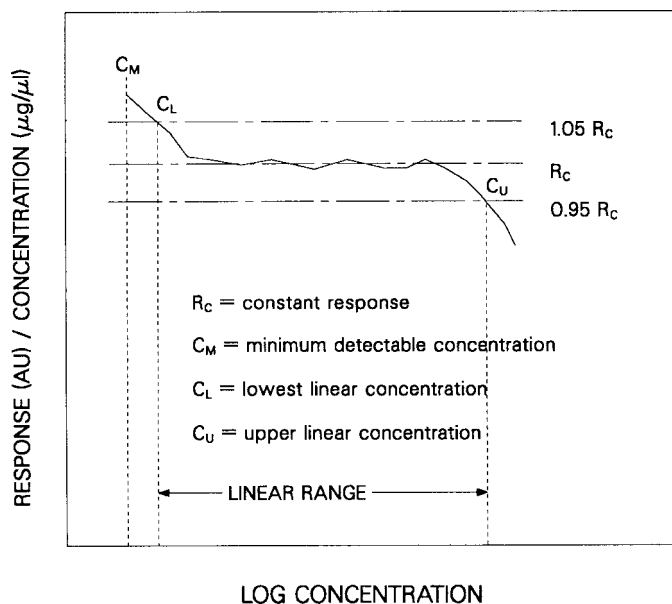


FIG. 1 Example of a Linearity Plot for a Photometric Detector

3.1.9 *static*—under conditions of no flow.

#### 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 of suitable grade<sup>5</sup> 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.

<sup>5</sup> 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 Methods of Measurement:

5.2.1 Connect a suitable device (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 PD that has a variable response time (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. 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 centimetres 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 centimetres 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 (see 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 through 5.2.3. Allow 15 min for the system to stabilize. Record

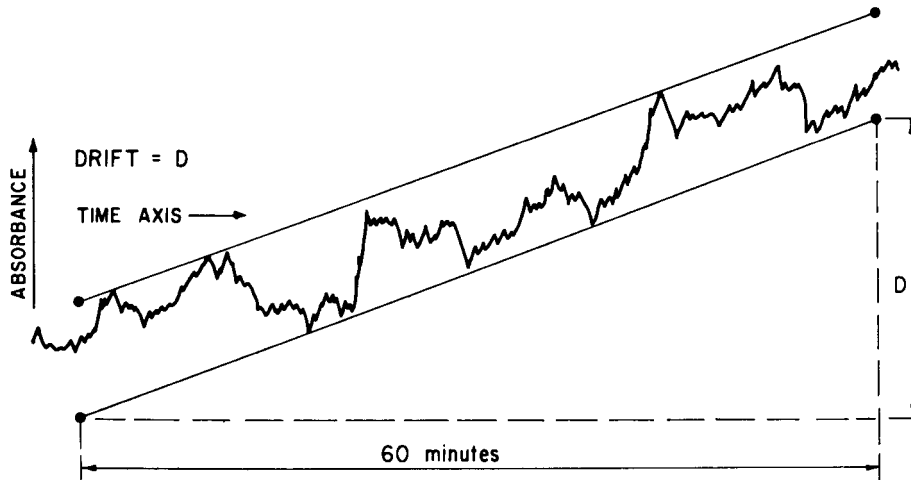
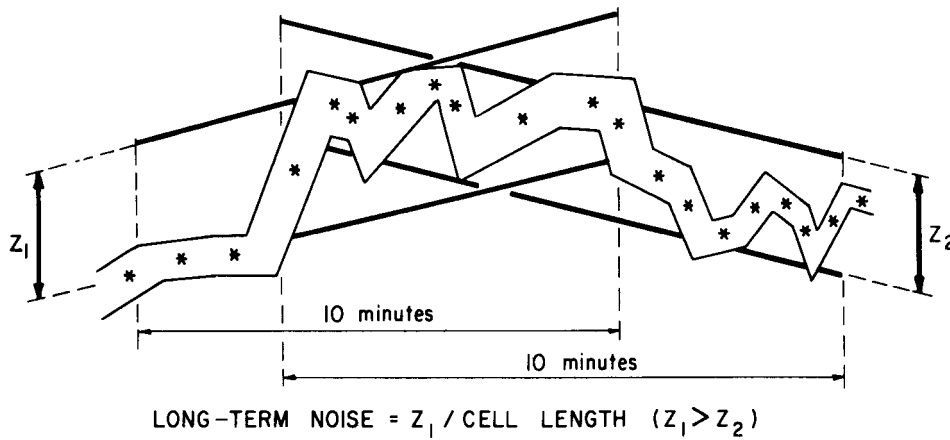
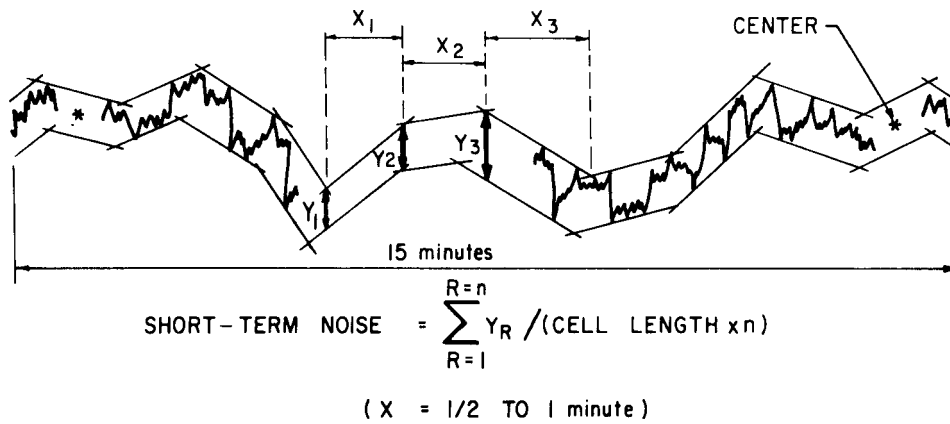


FIG. 2 Example for the Measurement of the Noise and Drift of a PD (Chart Recorder Output).

at least 1 h of signal under these flowing conditions, during which time the ambient temperature should not change by more than 2°C.

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. Minimum Detectability, Linear Range, and Calibration

6.1 *Methods of Measurement*—For the determination of the linear range of a PD, (12) for a specific substance, the response to that test substance must be determined. The following procedure is designed to provide a worst-case procedure.

6.1.1 Dissolve in methanol a suitable compound with an ultraviolet spectral absorbance that changes rapidly at the

wavelength of interest.<sup>6</sup> Choose a concentration that is expected to exceed the linear range, typically to give an absorbance above 2 AU. Dilute the solution accurately in a series to cover the linear range, that is, down to the minimum detectable concentration.<sup>7</sup> Rinse the sample cell with methanol and zero the detector with methanol in the cell. Rinse the cell with the solution of lowest concentration until a stable reading is obtained; usually rinsing the cell with 1 mL is sufficient. Record the detector output. After rinsing the syringe thoroughly with the next more concentrated solution, fill the cell with the solution from each dilution in turn. Obtain a minimum of five on-scale measurements. Measure under static conditions.

6.1.2 Calculate the ratio of detector response (AU) to concentration ( $\mu\text{g/mL}$ ) for each solution and plot these ratios versus log concentration (see Fig. 1). The region of linearity will define a horizontal line of constant response ratio. At higher concentrations, there will typically be a negative deviation from linearity, while at lower concentrations there may be deviation in either direction. Draw horizontal lines 5 % above and below the line of constant response ratio. The upper limit of linearity is the concentration at which the line of measured response ratio intersects one of the 5 % bracketing lines at the high concentration end. The lower limit of linearity is either the minimum detectable concentration (see 6.1.3) or the concentration at which the line of measured response ratio intersects one of the bracketing lines at the low concentration end, whichever is greater.

6.1.3 Determine the *minimum detectability* (minimum detectable concentration) of the test substance by calculating the concentration that would correspond to twice the static short-term noise. Specify the solute and solvent.

6.1.4 Calculate the ratio of the upper limit of linearity to the lower limit of linearity to give the *linear range* expressed as a number. As this procedure is a worst case situation, the linear range may be expected to be greater for compounds having a broad spectral band in the region of the chosen wavelength.

6.1.5 Plot or calculate the detector response (AU) versus concentrations ( $\mu\text{g/mL}$ ) for a test substance of known molar absorptivity to find the best-fit line through the origin. Calculate the molar absorptivity,  $\epsilon$ , of the test solution as follows:

$$\epsilon = \frac{\text{slope} \times MW}{b} \quad (1)$$

where:

*slope* = the slope of the linear portion of the plot, AU· $\mu\text{L}/\mu\text{g}$ ,  
*MW* = molecular weight, g/mole, and  
*b* = nominal cell length, cm, as specified by the manufacturer.

Compare the value of  $\epsilon$  obtained with an experimentally determined value or one from the literature (Note 3). Should the values differ by more than 5 %, the PD may require adjustment. Consult the manufacturer's directions.

NOTE 3—For example, the values of molar absorptivity for uracil in methanol are  $7.7 \times 10^3$  at 254 nm and  $1.42 \times 10^3$  at 280 nm; for potassium dichromate in 0.01 N sulfuric acid they are  $4.22 \times 10^3$  at 254 nm and  $3.60 \times 10^3$  at 280 nm.

## 7. Response Time

7.1 The response time of the detector may become significant when a short micro-particle column and a high-speed recorder are used. Also, it is possible, by using an intentionally slow response time, to reduce the observed noise and hence increase the apparent linear range. Although this would have little effect on broad peaks, the signal from narrow peaks would be significantly degraded. Measure at the highest and lowest values of the electronic filter if it is variable.

### 7.2 Method of Measurement:

7.2.1 The composition of the mobile phase is changed in a stepwise manner and the output signal is recorded on the highest-speed device available. If the recorder has a response time not significantly faster than the detector, only the response time of the detector-recorder combination will be obtained, as it would be when the combination is used to record chromatograms.

7.2.2 Set a flow rate of 2.0 mL/min.

7.2.3 A stepwise change may be obtained by means of a sample valve equipped with a 1-mL sample loop (or a loop having at least four times the total volume from detector inlet to outlet) connected between the pump and the detector. Observe the recorder trace and verify that a plateau has been reached. If no plateau is reached, a larger sample volume is required. This is likely to occur at high response times. Fill the sample loop with a solution of a concentration of test substance (see 6.1.1) in methanol sufficient to give a recorder detection of between 50 % and 95 % of full scale at suitable attenuation. The concentration should be within the linear range of the detector.

7.2.4 Repeat the measurement at 3.0 mL/min. If the value obtained is decreased from that at 2.0 mL/min, repeat the test at higher flow rates until a constant value is obtained.

7.2.5 Determine the time required for the signal to rise from 10 % to 90 % of the new equilibrium value from the recorder trace to give the *response time* (see Fig. 3). The chart speed should be fast enough to obtain an accurate measurement.

## 8. Refractive Index (RI) Sensitivity

8.1 Ideally, to minimize changes in baseline when running gradients, etc., UV detectors should be insensitive to changes in refractive index of the mobile phase. In this test the sensitivity to RI effects is determined by measuring the change in baseline of the detector when the cell is filled with methanol ( $n = 1.329$ ) and then with cyclohexane ( $n = 1.427$ ).

### 8.2 Materials Required:

#### 8.2.1 Chemicals:

8.2.1.1 *Cyclohexane*—HPLC grade,

8.2.1.2 *Methanol*—HPLC grade, and

8.2.1.3 *Ethanol or Denatured Ethanol*—Reagent Grade.

8.2.2 *Recorder*, accurately calibrated.

8.2.3 *Gas Tight Syringe*, 5 to 20 mL, fitted with appropriate connectors to give leak-proof seal onto detector inlet tubing.

### 8.3 Method of Measurement:

8.3.1 Switch on the detector and allow it to stabilize for at

<sup>6</sup> Benzaldehyde is suitable for testing at 214 and 254 nm, benzoic acid may be used at 280 nm.

<sup>7</sup> Stock solutions of 50 mg in 50 mL of L-C grade methanol are useful for this purpose. Suggested concentration ranges for the series of standards are 2.5 to 25  $\mu\text{g/mL}$  for benzaldehyde and 25 to 400  $\mu\text{g/mL}$  for benzoic acid.



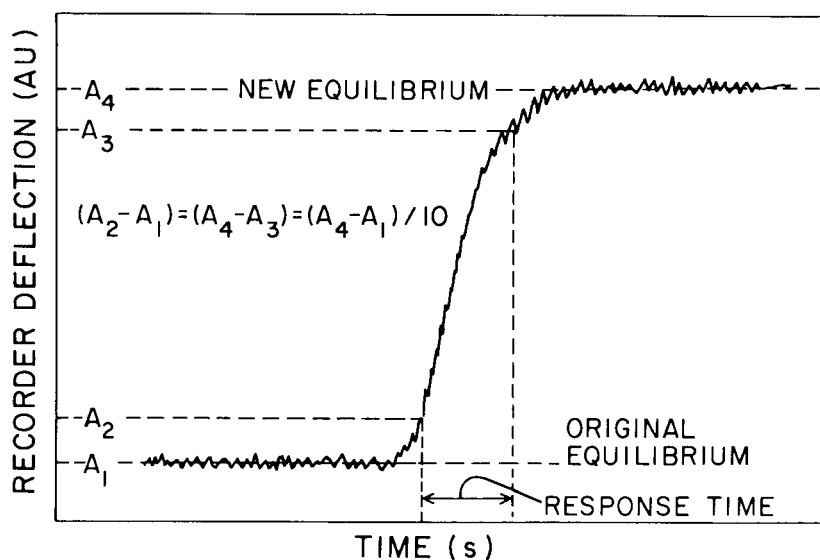


FIG. 3 Example for the Measurement of Response Time of a Photometric Detector.

least 1 h or the warm-up time specified by the manufacturer.

8.3.2 Set the wavelength to 280 nm and the detector/recorder output to 0.01 AUFS.

8.3.3 Set the chart speed to 1 cm/min.

8.3.4 Using the gas-tight syringe, fill the cell with methanol by passing at least 1 mL through the cell. Leave the syringe connected to the inlet tubing and seal the cell by capping the detector outlet tubing with an appropriate cap or plug.

8.3.5 Record at least 5 min of the baseline.

8.3.6 Remove the tubing cap or plug and repeat the procedure until the baseline does not change significantly (0.001 AU).

8.3.7 Remove the cap or plug, fill the syringe with ethanol or denatured ethanol, and flush the cell.

8.3.8 Clean, dry, and refill the syringe with cyclohexane. Repeat steps 8.3.4 to 8.3.6.

8.3.9 Measure and report the difference in the two baselines in AUs.

## 9. Further Description of Detector

9.1 For a more complete evaluation of a PD, factors other than those previously described are important. These are listed below, while typical values and units are listed in Table 1.

9.1.1 *Display Range of Attenuator*—The highest and lowest settings available at the detector output expressed in absorbance units full-scale detection (AUFS) for standard output voltage. This voltage is the millivolts full-scale deflection (mVFS) specified as standard for the recorder, so that the designated AU represents exactly full-scale detection of that recorder when zero signal is adjusted to recorder zero.

9.1.2 *Wavelength*—The central wavelength of the strongest spectral line passing through the sample cell.

9.1.3 *Bandpass*—The width of the spectral line at half maximum. For broad-band sources, this is determined by the bandpass of the optical filter.

9.1.4 *Cell Length*—The effective length of the fluid through which the light beam passes, measured along the cell axis.

9.1.5 *Cell Volume*—The volume of the effective part of the

cell, where the absorption of light takes place and where mixing may occur.

9.1.6 *Detector Volume*—The total volume of the detector between the inlet and outlet fittings. The inlet fitting shall be one capable of connecting directly to a chromatographic column; the outlet shall be capable of connecting to the inlet fitting of a second detector.

9.1.7 *Reference*:

9.1.7.1 In the case of a single-beam instrument, the detector is “reference—none.”

9.1.7.2 In the case of a double-beam instrument, the detector may have a reference cell. If so, this should be stated, or alternatively, “reference—air.”

9.1.7.3 If the ratio of light intensity is not 1:1 in balance on the sample and reference photodetectors (of a double-beam instrument), this should be stated.

9.1.8 *Monitor*—Presence or absence of a meter or other device to indicate the amount of light reaching the sample photodetector. State what the meter measures.

9.1.9 *Calibration Check*—Presence or absence of means to adjust the output of the detector to the specified absorbance value without use of an external device.

9.1.10 *Lamp Type*—Type of source lamp used in the detector.

9.1.11 *Estimated Average Lamp Life*—Average life of five or more lamps in continuous operation, usually to half intensity rather than failure.

9.1.12 *Pressure Limit*—Maximum operating pressure at which the cell is guaranteed to operate without leakage or hazard.

9.1.13 *Heat Exchanger*—The means, if any, by which the temperature of the influent is adjusted to a temperature similar to that of the detector cell.

9.1.14 *Wetted Materials of Cell*—All materials of the detector cell that are in contact with the mobile phase.

9.1.15 *Inlet Tube*—The material, length, and internal diameter of all tubing connecting the inlet fitting to the detector cell.

9.1.16 *Maximum Zero Offset*—The maximum amount by

**TABLE 1 Typical Values for Photometric LC Detectors**

Detector Characteristics	Units	Typical Values
<b>Measured Values</b>		
Static short-term noise	AU	$(0.5 \text{ to } 1.5) \times 10^{-4}$
Dynamic short-term noise	AU	$(0.5 \text{ to } 1) \times 10^{-4}$
Static long-term noise	AU	$(1 \text{ to } 4) \times 10^{-4}$
Dynamic long-term noise	AU	$(1 \text{ to } 5) \times 10^{-4}$
Static drift	AU/h	$(5 \text{ to } 10) \times 10^{-4}$
Dynamic drift	AU/h	$(2 \text{ to } 6) \times 10^{-4}$
Minimum detectability of (solute) in (solvent)	$\mu\text{g}/\mu\text{l}$	(depends on solution used)
Linear range	(ratio)	$(5 \text{ to } 10) \times 10^4$
Response time	s	1 to 5
<b>Specified Values, Dimensions, and Materials:</b>		
Display range of attenuator, min to max	AUFS/mVFS	0.01 to 2.6
Wavelength	nm	253.7, 280
Bandpass	nm	0.5 to 5
Cell length	mm	10
Cell volume	$\mu\text{l}$	8 to 25
Detector volume	$\mu\text{l}$	16 to 30
Reference	—	static air
Monitor	—	meter (reading millivolts)
Calibration check	—	none
Lamp type	—	low-pressure mercury
Estimated average lamp life	h	8500
Pressure limit	kPa <sup>A</sup>	1500
Heat exchanger	—	50-mm inlet tubing contacting cell
Wetted materials of cell	—	Type 316 stainless steel, TFE-fluorocarbon, quartz
Inlet tube: material, length, ID	—, mm, mm	Type 316 stainless steel, 100 mm, 0.1 to 0.3 mm
Max zero offset: fine, coarse	AU	15, $135 \times \text{FSD}^B @ 0.01 \text{ AUFS}$
Photodetector type	—	photomultiplier, photodiode
Stray light filter	—	none, interference filter

<sup>A</sup> 1 kPa = 0.15 psi.

<sup>B</sup> FSD = full-scale deflection.

which the zero value of the detector can be changed (a) by the fine control and (b) by the coarse and fine controls together.

#### 9.1.17 Type of Photodetector.

9.1.18 Stray Light Filter—If present, indicate type or types and respective bandpass.

### 10. Typical Values

10.1 The detector characteristics given in Section 9, and

measured under the conditions recommended, may be expected to fall near the values or ranges given in Table 1. The table indicates the units or way in which the characteristics should be expressed.

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