



Designation: E 579 – 84 (Reapproved 1998)

Standard Test Method for Limit of Detection of Fluorescence of Quinine Sulfate¹

This standard is issued under the fixed designation E 579; 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 test method employs the signal-to-noise ratio theory to determine the sensitivity of a fluorescence measuring system in testing for the limit of detection (LOD) of quinine sulfate. The results obtained with quinine sulfate are suitable for specifying instrument performance on samples having excitation and fluorescence bands wider than 10 nm.

1.1.1 This test method is not intended to be used as (1) a rigorous test of performance of instrumentation, or (2), to intercompare the quantitative performance of instruments of different design.

1.2 This test method has been applied to fluorescence-measuring systems utilizing continuous and low-energy excitation sources (for example, an excitation source of 150-W electrical input or less).² There is no assurance that extremely intense illumination will not cause photodecomposition³ of the compound suggested in this test method. For this reason, it is recommended that this test method not be indiscriminately employed with high intensity light sources. This test method is not intended to determine minimum detectable amounts of other materials. If this test method is extended to employ other chemical substances, the user should be aware of the possibility that these other substances may undergo decomposition or adsorption onto containers.

1.3 The practical lower limit of this test method is 1 ng of quinine sulfate per mL (one part per billion; 1 ppb).

1.4 *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:

¹ This test method is under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.06 on Molecular Luminescence.

Current edition approved March 30, 1984. Published May 1984. Originally published as E 579 – 76. Last previous edition E 579 – 76.

² Except laser-excited

³ Lukasiewicz, R. J., and Fitzgerald, J. M., *Analytical Chemistry*, ANCHA, Vol 45, 1973, p. 511.

E 578 Test Method for Linearity of Fluorescence Measuring Systems⁴

3. Summary of Test Method

3.1 To measure the concentration corresponding to the limit of detection, the amplification is increased until the noise observed with pure solvent in the sample cell is large enough to measure on the amplifier readout. The test solution is then diluted until readings on both the test solution and pure solvent can be read at the same amplification setting. The concentration corresponding to the limit of detection is that at which the noise, multiplied by three, is equal to the signal. The concentration of the sample corresponding to the limit of detection arrived at by this technique is indicative of the inherent noise in the instrument.

3.2 This test for limit of detection requires an instrument to be in good condition: stable, free of extraneous noise, electrical pickup, and internal stray light. The sample space must be covered to exclude room light. The instrument should be operated according to the manufacturer's recommendations, or, if they are modified, the modifications must be applied consistently to the test for limit of detection and to the analysis for which the test is a requirement, so that levels of performance are comparable for both. All modifications must be included in the report.

NOTE 1—To obtain the lowest reading (the best instrumental response) for the limit of detection of fluorescent material, a number of precautions must be taken. The condition and position of the sample cell are most important. The cell must be free of scratches and marks that scatter light into the emission monochromator. Only spectral grade chemicals and solvents (including water) should be used. Dilute solutions should be made, just before use, from concentrated stock solutions. All samples used must be maintained at the same temperature to obviate effects due to temperature coefficients.

4. Significance and Use

4.1 When determining the limiting detectable concentration of a fluorescent substance, it is usually necessary to increase the amplification of a photoelectric instrument to a point where noise (that is, random fluctuations of the system) becomes

⁴ *Annual Book of ASTM Standards*, Vol 03.06.

apparent. This noise will be superimposed upon the signal from the sample; it arises from several sources, such as instability in the zero point, tube noise, and statistical variations in the photoelectric current.

4.2 In molecular fluorescence spectroscopy, the limit of detection for the sample will be determined by the limiting signal-to-noise ratio, S/N , where the signal, S , is the difference between readings obtained with the sample and blank solutions, and N is the total root-mean-square (rms) noise due to all sources of noise, including random errors arising due to cuvette position. The limit of detection for the sample will be given by the instrument readings that give a signal equal to three times the rms value of the noise.

NOTE 2—Factors other than noise affecting the sample concentration corresponding to the limit of detection include: the spectral bandwidths of the excitation and emission monochromators, the intensity of the exciting light that can be concentrated on the sample, the fraction of the fluorescence that can be utilized by the detection system, the response time of the detection system, and the purity of the solvent. The size and arrangement of the sample container with respect to the light beams are also important, as they affect both the desired signal and the extraneous signal that only contributes noise.

NOTE 3—The value of rms noise can be obtained by calculating the standard deviation of a series of readings of the signal from the sample as follows:

$$\text{rms} = \sqrt{\sum(\bar{X} - X)^2/(n - 1)} \quad (1)$$

where:

$\bar{X}$ = mean of the series of readings,
 X = value of the individual reading, and
 n = number of readings.

Alternatively, rms noise may be estimated by noting the extreme differences between the members of a series of readings (peak-to-peak noise) and dividing by a factor that is usually taken to be 5.^{5,6}

5. Reagents

5.1 Prepare the test solution of quinine sulfate dihydrate ($\text{C}_{20}\text{H}_{22}\text{O}_2\text{N}_2$) $\cdot\text{H}_2\text{SO}_4\cdot 2\text{H}_2\text{O}$ in accordance with Test Method E 578.

6. Procedure

6.1 Adjust the fluorescence-measuring system for normal operating conditions. The widest excitation and emission bandwidth available on the instrument should be used (not to exceed 40 nm).

6.2 Set the excitation wavelength and emission wavelength in accordance with Test Method E 578. For quinine sulfate, the peak wavelengths will be approximately 350 and 450 nm, respectively.

NOTE 4—In some fluorescence measuring systems, it may not be possible to adjust excitation or emission wavelengths to obtain the maximum fluorescence of quinine. However, users of such instruments should be aware of the Raman scatter phenomenon due to solvent alone. Such Raman scatter may contribute significantly and independently to noise, blank, or test solution readings.

⁵ Blair, E. J., *Introduction to Chemical Instrumentation*, McGraw-Hill, New York, NY, 1962.

⁶ Landon, V. D. *Proceedings of the I. R. E. and Waves and Electrons*, PIWEB, Vol 29, 1941, p. 50.

6.3 Set the time constant, if adjustable, to the value nearest to 1 s, so that it is equivalent to a 98 % response time of 4 s.

6.4 Put the pure solvent, in this case 0.1 N H_2SO_4 , in the sample cell and increase the amplification of the fluorescence-measuring system to maximum or until the peak-to-peak noise is approximately 5 % of full scale on the readout device. Set the zero adjustment to bring the reading of the solvent to slightly above zero on the scale. Measure the signal from at least ten independent readings, removing and reinserting the sample cell after each reading. The average of these ten signals, $\bar{B}$, is used in 7.1.

NOTE 5—In some cases, removal and reinsertion of the sample cell may not be feasible, such as, in process control (continuous flow analysis) or chromatographic column effluent monitors. With such instrumentation, emission from 10 aliquots of solvent and 10 aliquots of test solution should be measured. In other instruments with analog (chart paper) output, it will be necessary to select 10 record segments for measurements of signals from the solvent and the test solution.

6.5 Replace the pure solvent with a dilute test solution (1 ng/mL or greater) in the same cell. Note the readings of the signal from this sample. The meter readings should be less than 100 % and greater than 10 % of full-scale deflection. If the signal, s , resulting for this test solution does not fall within these limits, replace the test solution with a more concentrated test solution, if applicable. Repeat the measurement of (s) ten times as in step 6.4, removing and reinserting the sample cell after each reading. Average the 10 measurements of s to obtain the average, $\bar{S}$.

7. Calculation

7.1 Take the difference between the average signal ($\bar{S}$) resulting from the sample solution measurements and ($\bar{B}$), that resulting from the average of the ten readings of the blank solution. This is the *net* signal (S) due to the substance in the solvent, used in the equation shown in 7.2.

7.2 Calculate the LOD as follows:

$$\text{LOD} = (\text{sample concentration}/S) \times (\text{rms noise} \times 3) \quad (2)$$

7.2.1 Report the average LOD.

8. Report

8.1 Report the LOD of quinine sulfate in nanograms per millilitre.

8.2 If the manufacturer's recommendations for the operation of the instrument were modified for the performance of this test, report all such modifications.

9. Precision and Bias

9.1 The precision of this test method is limited by the root-mean-square noise in the fluorescence measuring system when the amplification of peak-to-peak noise from 0.1 N H_2SO_4 is approximately 5 % of the full scale of the read-out device.

9.2 This test method is not intended to be used as (1) a rigorous test of performance of instrumentation, or (2), to intercompare the quantitative performance of instruments of different design. Therefore, no statement of bias can be made.