Designation: E579 – 04 (Reapproved 2015)

Standard Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution[1]

This standard is issued under the fixed designation E579; 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 to determine the sensitivity of a fluorescence measuring system in testing for the limit of detection (LOD) of quinine sulfate dihydrate in solution. The results obtained with quinine sulfate dihydrate in solution are suitable for specifying instrument performance on samples having excitation and fluorescence bands wider than 10 nm at or near room temperature.

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. Intercomparison of the LOD between instruments is commonly expressed as the ratio of the water Raman peak intensity to the root-mean-square (rms) noise as measured on a fluorometer using an excitation wavelength of 350 nm This test method uses the excitation and emission peak wavelengths for quinine sulfate dihydrate in solution, which are approximately 350 nm and 450 nm, respectively.

1.2 This test method has been applied to fluorescence-measuring systems utilizing non-laser, low-energy excitation sources. There is no assurance that extremely intense illumination will not cause photodecomposition[2] 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 A typical LOD for conventional fluorometers using this test method is 1 ng of quinine sulfate per mL.

1.4 The suggested shelf life of a 1 mg/mL stock solution of quinine sulfate dihydrate is three months, when stored in the dark in a stoppered glass bottle.

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 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:*[3]

E578 Test Method for Linearity of Fluorescence Measuring Systems

3. Summary of Test Method

3.1 To measure the concentration corresponding to the LOD, the fluorescence intensity scale and gain on the detector are adjusted such that noise observed with pure solvent in the sample cell is large enough to measure. The test solution is then diluted until readings on both the test solution and pure solvent can be read at the same intensity, scale, and instrument settings. The concentration corresponding to the limit of detection is that at which the noise intensity, multiplied by three, is equal to the signal intensity.

3.2 This test for limit of detection requires an instrument to meet the following conditions: 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 outlined in Section 8.

[1] This test method 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] Lukasiewicz, R. J., and Fitzgerald, J. M., *Analytical Chemistry*, ANCHA, Vol 45, 1973, p. 511.

[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.

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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 quality, condition, and position of the sample cell are most important. The cell must be made of fused silica that does not fluoresce at the excitation wavelength and be free of scratches and marks that scatter light into the fluorescence detection system. Only spectral grade chemicals and solvents (including water) that do not fluoresce should be used.[4] Dilute solutions of quinine sulfate dihydrate 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 fluctuations. The average temperature coefficient for fluorescence intensity in the temperature range from 16 – 35°C is –0.62 %/°C at 450 nm for 1 µg/mL quinine sulfate dihydrate in 0.1 mol/L $HClO_4$.[5]

4. Significance and Use

4.1 When determining the limiting detectable concentration of a fluorescent substance, it is usually necessary to increase the readout scale of a photoelectric instrument to a point where noise (that is, random fluctuations of the system) becomes apparent. This noise will be superimposed upon the signal from the sample.

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. 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 collected 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 (*N*) can be obtained by calculating the standard deviation of a series of readings of the signal from the sample at the peak emission wavelength at approximately 450 nm 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.[6, 7]

5. Reagents

5.1 Prepare a stock solution of quinine sulfate dihydrate $(C_{20}H_{22}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ by transferring 0.100 g of high purity crystalline dihydrate of quinine sulfate[8] into a 100-mL volumetric flask and fill the flask to volume using either 0.1 mol/L sulfuric acid or 0.1 mol/L perchloric acid as the solvent. This solution contains 1 mg/mL of quinine sulfate dihydrate.

NOTE 4—Either 0.1 mol/L sulfuric acid or 0.1 mol/L perchloric acid can be used as a solvent with quinine sulfate dihydrate, but the solvent that is chosen must also be used as the blank. Take note that the quantum yield of quinine sulfate dihydrate in solution has been shown to be about 13 % smaller in 0.1 mol/L sulfuric acid than in 0.1 mol/L perchloric acid, which will result in a corresponding increase in the concentration of quinine sulfate dihydrate in 0.1 mol/L sulfuric acid versus that in 0.1 mol/L perchloric acid at the LOD for a particular instrument.

5.2 Make serial dilutions by diluting aliquots of the stock solution and successive solutions to ten times their volume with the solvent. Repeat this process until the desired concentration is obtained. The sixth successive dilution will result in a concentration of 1 ng/mL.

5.3 Any fluorescence from the pure solvent will interfere with the limit of detection measurement. The solvent should be tested for fluorescence before being used with this method. To test for fluorescence, follow the procedures given in sections 6.1 to 6.5, but replace the blank with an empty sample cell, that is, just air in the cell, and replace the dilute test solution with the blank.

5.4 Calculate $\bar{S}$ and $\bar{B}$, the average signal of the blank and empty cell , respectively, and the rms noise of the signal from the empty cell, as described in 7.1. If $\bar{S}$ is greater than $\bar{B}$ by more than three times the rms noise of the empty cell signal, then fluorescence from the solvent may be present.

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 E578. For quinine sulfate dihydrate in solution, the peak wavelengths will be approximately 350 and 450 nm, respectively.

NOTE 5—In some fluorescence measuring systems, it may not be possible to adjust excitation or emission wavelengths to obtain the maximum fluorescence of quinine sulfate dihydrate in solution. 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.

6.3 Set the signal integration time to 1 s, or the instrumental equivalent.

6.4 Put the pure solvent in the sample cell and adjust instrument settings such that the peak-to-peak noise is approximately 5 % of full range of the instrument at these settings. This readout scale is referred to as "full scale" in all sections that follow. Measure the signal from the blank for 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.

[4] The procedure used to recognize fluorescence in a solvent is given in 6.3 and 6.4.

[5] Velapoldi, R. A., and Mielenz, K. D., *NBS Special Publication* 260–64, 1980, p. 60.

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

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

[8] National Institute of Standards and Technology SRM 936a, or the equivalent.

NOTE 6—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.

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. If the signal, s, resulting for this test solution does not fall within these limits, replace the test solution with a 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 $\bar{S}$, the average signal resulting from the sample solution measurements and $\bar{B}$, that resulting from the average of the ten readings of the blank solution. This is S the *net* signal due to the substance in the solvent.

$$S = \bar{S} - \bar{B} \tag{2}$$

7.2 Calculate the LOD as follows:

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

7.2.1 Report the average LOD.

8. Report

8.1 Report the LOD of quinine sulfate dihydrate in solution 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, these modifications should be noted.

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 peak-to-peak noise from the blank is amplified.

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

10. Keywords

10.1 fluorescence spectrometers; molecular luminescence; molecular spectroscopy

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