TECHNICAL NOTE

Fluorescence Spectroscopy

Limits of Detection of Quinine Sulfate Using the FL 6500 and FL 8500 Fluorimeters According to ASTM E579-04

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Introduction

The Limit of detection (LOD) is defined as the lowest concentration of analyte which can be distinguished from the signal obtained from the blank, or absence of the analyte, with reasonable certainty using a specified analytical method. The LOD is, therefore, also typically defined as the analyte concentration that gives rise to a signal which is equal to three times the standard deviation of the noise.[1-3]

This technical note demonstrates the limits of detection of the FL6500 and FL8500 fluorimeters using quinine sulfate in 0.1 M sulfuric acid, according to ASTM E579-04 (2015). This test method employs the signal-to-noise ratio to determine the sensitivity of a fluorimeter in testing the LOD of quinine sulfate dihydrate in solution. The LOD may be calculated using Equation 1, where $\overline{S}$ is the average (n = 10) signal of the sample solution measurement, $\overline{B}$ is the average (n = 10) reading obtained from the blank, and the RMS (root-mean-square) noise obtained by calculating the standard deviation of the blank readings.[3]

$$LOD = \frac{(sample\ concentration)}{\overline{S} - \overline{B}} (3 \times RMS\ noise) \quad (1)$$

The limits of detection reported in this technical note are only achievable using quinine sulfate with the instrument settings specified. Detection limits will vary depending upon the instrument settings (including excitation and emission wavelengths) and the fluorophore used.[3] By way of example, fluorescein has a higher quantum yield than quinine sulfate, thus lower detection limits would be expected for fluorescein.[4]

ASTM Standard Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution (E579 – 04)

ASTM designation E579–04 employs the signal-to-noise ratio to determine the sensitivity of a fluorimeter in testing for the LOD of quinine sulfate dihydrate in solution. A typical LOD for conventional fluorimeters using this test method is 1 ng/mL quinine sulfate. ASTM E579-04 specifies that the test method is not intended to be used as a rigorous test of instrument performance, or as an inter-comparison of the quantitative performance of instruments of different design. Instead, inter-comparison of the LOD between instruments is typically carried out by measuring the signal-to-noise ratio of the Raman peak intensity of pure water to the root-mean-square (rms) noise using a fixed excitation wavelength of 350 nm.[3]

The PerkinElmer FL6500 and FL8500 fluorimeters use PMT detectors. Noise in a spectrometer measurement includes two distinct sources. Thermal noise, also known as "dark" noise, is unavoidable and a result of random thermal motion of electrons conducted through the detector.[5] This thermal noise occurs regardless of the voltage applied and is solely dependent on temperature. Thermal noise, like other forms of noise, is random in nature, thus dark signal introduces noise into the spectrum and some uncertainty into each measurement. Shot noise, also known as counting noise, arises from the statistical variation (Poisson distribution) in the number of photons incident on the detector. This type of noise is signal dependant, becoming more significant as the voltage is increased in an effort to increase the signal intensity.[5-8]

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Other factors, other than noise, which may influence the value for the limit of detection include solvent purity, the spectral bandwidth of the excitation and emission monochromators, the intensity of the exciting light which is directed onto the sample, and the fraction of the fluorescence collected by the detector.[3]

Method

Fluorescence grade quinine sulfate dihydrate (≥ 98 %) (Mr = 391.47 g/mol) and ACS reagent grade sulfuric acid (95 %) were obtained from Merck (previously Sigma-Aldrich). ASTM E579-04 specifies a dilute test solution of 1 ng/mL or greater should be used. A stock solution (2.55 mM / 1 mg/mL) of quinine sulfate in 0.1 M sulfuric acid was prepared by mass. From this solution, serial dilutions of the stock were prepared using a 10-fold dilution factor. The sixth successive solution had a concentration of 2.55 nM (1 ng/mL).

Replicate fluorescence intensity measurements (n = 10) of the blank (0.1 M H_2SO_4) and 2.55 nM (1 ng/mL) quinine sulfate solution were achieved using the PerkinElmer FL6500 and FL8500 (Figure 1) with the single cell holder (Figure 2) and 10 x 10 mm pathlength quartz cuvettes. Independent readings were carried out by removing and reinserting the blank and sample cell after each scan. Spectrum® FL software was used with the instrument settings specified for the FL6500 and FL8500 in Table 1 and 2, respectively. The 'Transform' button in Spectrum FL software allows methods to be converted between the FL6500 (pulse xenon illumination source) and the FL8500 (continuous xenon arc illumination source) instruments.

Figure 1. PerkinElmer FL6500 (top) and FL8500 (bottom).

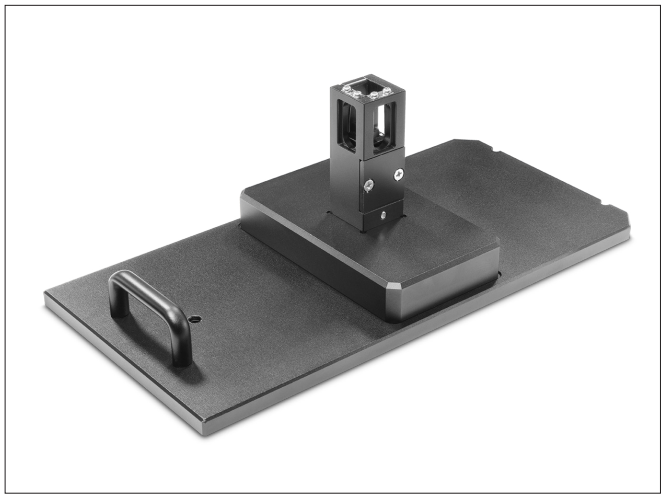

Figure 2. Standard single cell holder accessory (P/N: N4201010) in the FL6500 and FL8500 Fluorimeters.

Table 1. FL6500 instrument settings for quinine sulfate.

Scan Settings		
Source	Source mode	Pulse
	Excitation correction	On
	Initial dark	On
	Power (kW)	80
	Frequency (Hz)	100
Excitation	Excitation wavelength (nm)	350
	Slit width (nm)	10
	Filter	Air
Emission	Wavelength range (nm)	375-550
	Slit width (nm)	20
	Scan speed (nm/min)	240
	Accumulation number	1
	Filter	Air
Acquisition	Voltage (V)	550
	Response width (nm)	5
	Emission correction	On
	Gain	x1

Table 2. FL8500 instrument settings for quinine sulfate.

Scan Settings		
Source	Source mode	Continuous
	Excitation correction	On
	Initial dark	On
	Chopper (Hz)	10
Excitation	Excitation wavelength (nm)	350
	Slit width (nm)	10
	Filter	Air
Emission	Wavelength range (nm)	375-550
	Slit width (nm)	20
	Scan speed (nm/min)	240
	Accumulation number	1
	Filter	Air
Acquisition	Voltage (V)	550
	Response width (nm)	5
	Emission correction	On
	Gain	x1

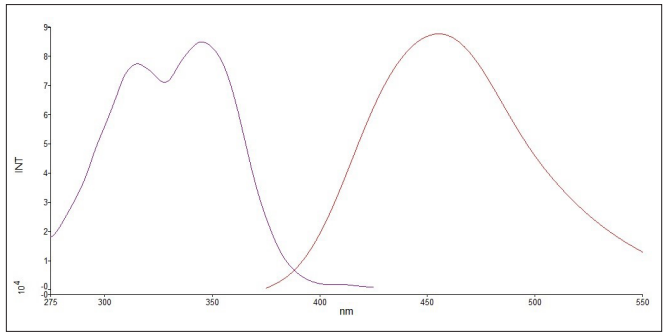

Figure 3. Excitation (left) and emission (right) spectra of 1 µg/mL quinine sulfate in 0.1 M H_2SO_4 using the FL6500.

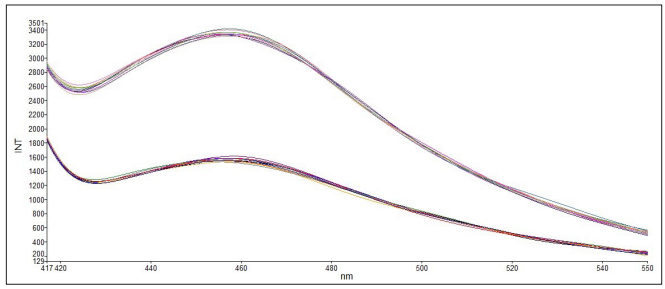

Figure 4. Ten replicate spectra of 1 ng/mL quinine sulfate (top) and blank (0.1 M H_2SO_4) (bottom) using the FL6500.

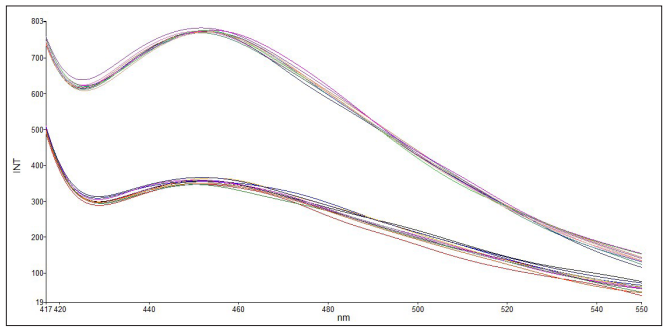

Figure 5. Ten replicate spectra of 1 ng/mL quinine sulfate (top) and blank (0.1 M H_2SO_4) (bottom) using the FL8500.

Results

Excitation and emission spectra of quinine sulfate in 0.1 M H_2SO_4 using the FL6500 are shown in Figure 3. Figures 4 and 5 demonstrate the emission spectra of the blank and 1 ng/mL quinine sulfate solution using the FL6500 (Figure 4) and the FL8500 (Figure 5). The LODs for the FL6500 and FL8500, using Equation 1 and the specified instrument settings in Tables 1 and 2, were determined to be 0.13 nM (0.05 ng/mL) and 0.11 nM (0.04 ng/mL) quinine sulfate, respectively.

Table 3. Limits of detection (nM and ng/mL) of quinine sulfate using the FL6500 and FL8500 under the instrument settings specified in Table 1 and 2, respectively.

	Average (n=10) Fluorescence Intensity ± Standard Deviation		Quinine Sulfate Limit of Detection (LOD)	
Instrument	**Blank (0.1 M H_2SO_4)**	**2.55 nM (1 ng/mL) Quinine Sulfate**	**nM**	**ng/mL**
FL6500 (Pulse)	1544.57 ± 29.03	3334.43 ± 36.40	0.13	0.05
FL8500 (Continuous)	365.01 ± 5.67	786.57 ± 5.57	0.11	0.04

Conclusion

The PerkinElmer FL6500 and FL8500 fluorimeters demonstrate good sensitivity, in comparison with ASTM E579-04, in testing for the limit of detection of quinine sulfate dihydrate in solution. The LODs of quinine sulfate in 0.1 M sulfuric acid using the FL6500 and FL8500 were determined to be 0.13 and 0.11 nM (0.05 and 0.04 ng/mL), respectively. These reported limits of detection are only achievable using quinine sulfate with the instrument settings specified.

References

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