APPLICATION NOTE

Fluorescence Spectroscopy

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Determination of Quinine in Tonic Water Using Fluorescence Spectroscopy

Introduction

Quinine, first isolated from the bark of the South American Cinchona tree in 1820, is an effective anti-malarial drug and used as a flavouring agent in carbonated beverages such as tonic water. Originally, quinine mixed with carbonated water was heavily consumed in the 19th century as a preventative measure for malaria. However, due to the distinctive bitter taste, it was quickly discovered that mixing the drink with gin made it much more palatable, giving rise to the iconic gin and tonic cocktail (albeit a potent precursor).[1-3] The use of quinine as a flavour in carbonated beverages is now limited by the U.S. Food and Drug Administration (FDA) to 83 ppm, with most commercial tonic waters containing about 25 - 60 ppm quinine, significantly lower concentrations than in the 19th century.[4,5]

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Fluorescence spectroscopy is a very sensitive and selective analytical technique for detecting and measuring trace amounts of organic compounds. The selective nature of this technique arises because each compound is characterized by an excitation and emission wavelength. This application demonstrates the use of fluorescence spectroscopy, with the PerkinElmer FL6500, for the simple determination of quinine in tonic water, providing a more sensitive and selective technique than absorption spectroscopic techniques.

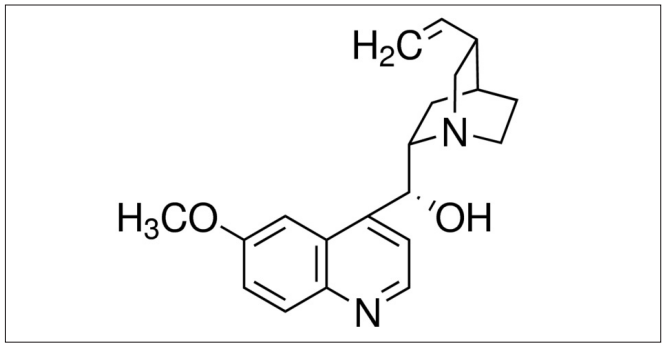

Figure 1. Chemical structure of Quinine (recreated using PerkinElmer ChemDraw®).

Quinine Fluorescence

Fluorescence spectroscopy can be used to quantify the concentration of quinine in tonic water, providing the instrument conditions remain constant and the quinine is in a dilute acid solution. The chloride ion is the only normal interfering species as it quenches quinine fluorescence. Although this has been found to be negligible, providing the concentration of the chloride ion in tonic water is below 0.4 mM, which it usually is. In dilute sulfuric acid, quinine has two analytically useful excitation wavelengths; 250 and 350 nm. However, the wavelength of maximum fluorescence (emission wavelength) is always 450 nm, regardless of the excitation wavelength used. The fluorescence intensity will vary depending on the relative strength of absorption.[6,7]

Excitation and Emission peaks observed for quinine analysis:

- First excitation peak at 250 nm, which corresponds to an $S_0 \rightarrow S_2^*$ transition
- Second excitation peak at 350 nm, which corresponds to an $S_0 \rightarrow S_1^*$ transition
- Single emission peak at 450 nm, which corresponds to the $S_1 \rightarrow S_0$ transition

Only one emission peak is observed because, following light absorption and subsequent excitation to higher energy levels, quinine undergoes both fluorescence and internal conversion. Fluorescence is a radiative process which occurs following excitation and thermal degradation (relaxation) to the ground vibrational state of the excited electronic state. The radiation emitted on return to the ground electronic state has a longer wavelength and, thus, lower energy than that of the absorbed radiation. Internal conversion, on the other hand, is a non-radiative and very efficient process in which energy is dissipated if vibrational energy levels of different electronic energy states with the same multiplicity strongly overlap (e.g. S_1^* and S_2^*). Internal conversion is very efficient between the two electronically excited states of quinine and, therefore, only one emission at 450 nm is observed.[7]

Experimental

Calibration

Anhydrous fluorescence grade quinine (≥ 98.0%) and ACS reagent grade sulphuric acid (95%) were obtained from Merck (previously Sigma-Aldrich). The quinine stock solution (1000 ppm) was prepared in a pre-weighed 50 mL polypropylene vial by diluting an accurately weighed amount of quinine (0.05 g) with 0.05 M H_2SO_4. Quinine sulfate (0.0604 g) may also be used. From this stock solution, a working solution (100 mg/L quinine) was prepared in 0.05 M H_2SO_4. Seven calibration standards were prepared by mass from the working solution over the calibration range 0.05 – 1.5 ppm by dilution with 0.05 M H_2SO_4.

All standards and samples were measured using the PerkinElmer FL6500 Fluorescence Spectrometer (Figure 2) with the Single Cell Accessory and 10 x 10 mm quartz fluorescence cuvettes. Background correction was carried out using a blank solution of 0.05 M H_2SO_4. Spectrum® FL software was used with the instrument settings specified in Table 1. The Spectrum FL software provides a choice of quantification methods, being Scan Quant which uses a defined emission wavelength range, or Wavelength Quant which uses a specific emission wavelength. In the case of this application, the Scan Quant function was used between 360 - 580 nm to gain spectra of the calibration standards.

Figure 2. PerkinElmer FL6500 Fluorescence Spectrometer.

Table 1. Operating parameters used for the FL6500 Fluorescence Spectrometer.

Instrument Settings		
Source	Flash power	80 kW
	Frequency	100 Hz
	Excitation filter	Air
Excitation	Wavelength	350 nm
	Slit width	2.5 nm
	Correction	On
Emission	Wavelength scan	360 – 580 nm
	Slit width	2.5 nm
	Correction	On
	Filter	320 nm
	Scan speed	240 nm/min
Acquisition	Response width	20 nm
	PMT voltage	550 V (medium)
	PMT gain	x1

Preparation of Commercial Tonic Water Samples

Four different brands of tonic water were bought from a local supermarket and analyzed as unknown samples. A small aliquot of each tonic water sample was initially vigorously shaken in a glass bottle to remove the dissolved carbon dioxide. From these de-gassed tonic water samples, 0.5 ml was taken and diluted to a final volume of 50 mL in 0.05 M H_2SO_4, providing a 100-fold dilution factor and final quinine concentrations which lay around the middle of the calibration curve. As per the calibration standards, samples were prepared accurately by mass and transferred into separate 10 x 10 mm quartz fluorescence cuvettes to be analysed.

Results

Calibration

Spectra and calibration curve of the quinine calibration standards are shown in Figures 3 and 4, respectively. The linear regression coefficient (R^2) obtained from the calibration curve, displayed in Figure 4, is greater than 0.999 indicating a high level of correlation.

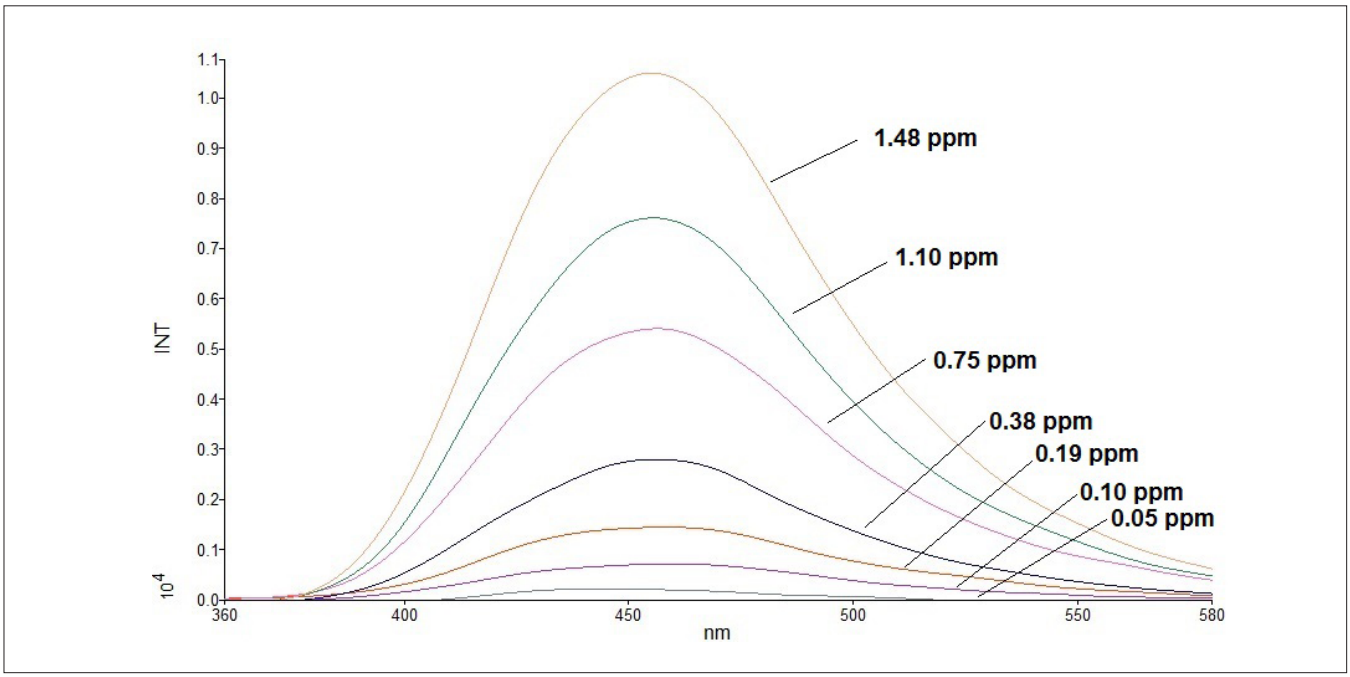

Figure 3. Spectra of quinine calibration standards in 0.05 M H_2SO_4 using the FL6500.

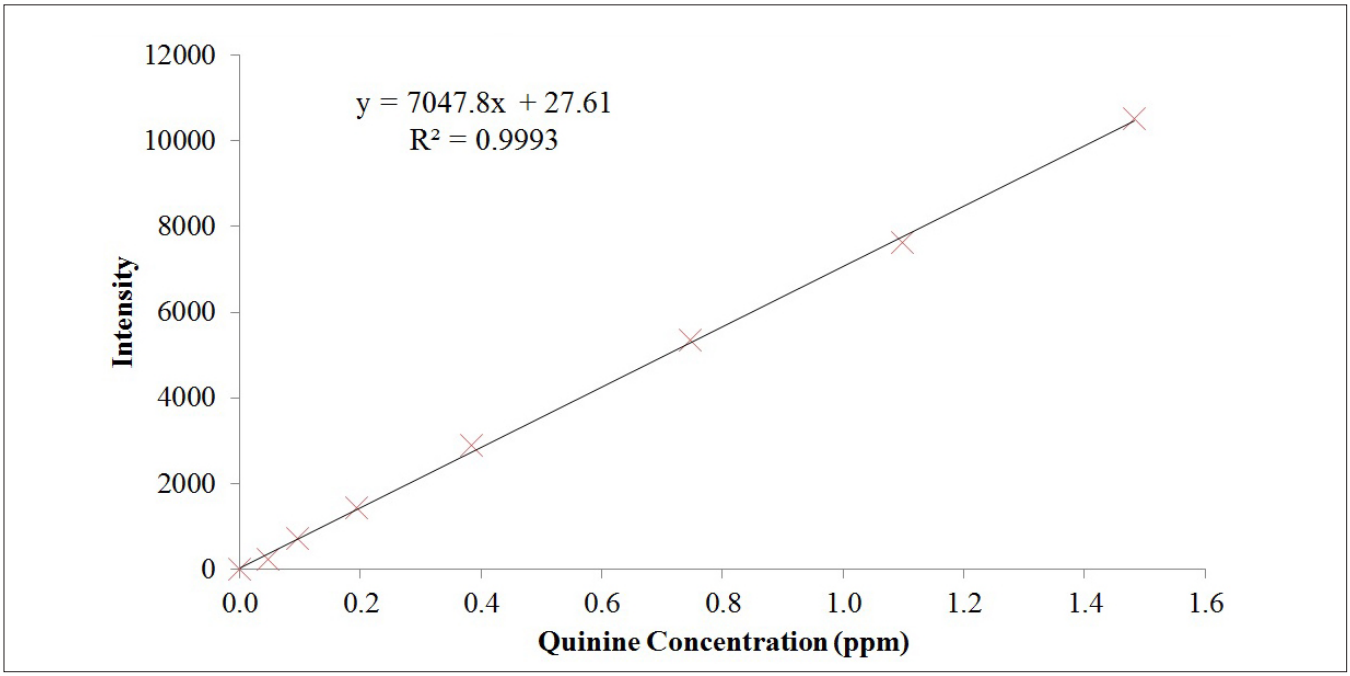

Figure 4. Calibration curve (including the 0.05 M H_2SO_4 blank) for the determination of quinine in tonic water samples using the FL6500.

Tonic Water Sample Measurement

The quinine concentrations observed in the four commercially available tonic waters analyzed are shown in Table 2. The measured concentrations were multiplied by 100 to obtain the actual concentration due to the 100-fold dilution factor. The concentrations observed differed between each of the brands and were between 57 and 80 ppm, all within the maximum level of 83 ppm permitted by the FDA. Sample 2 had a slightly higher value than the others, but nevertheless still within the permitted level.

Table 2. Dilution factor corrected quinine concentrations of four different commercial tonic water brands using the FL6500.

Tonic Water Sample	Quinine Concentration (ppm)
Sample 1 tonic water	61.0
Sample 2 tonic water	79.9
Sample 3 tonic water	57.0
Sample 4 tonic water	64.8

Method Validation

To evaluate the levels of accuracy and repeatability of this method, four commercially available tonic waters, two quality control (QC) quinine standards (29 ppm and 59 ppm), and spike recovery studies were used. The QCs were prepared independently of the calibration standards. It is important to note that the 100-fold dilution factor was included in determining the final quinine concentration and in statistical analysis.

The level of precision, in terms of repeatability, was assessed by the standard deviation and reported as the percentage relative standard deviation (% RSD). The repeatability of measurements was determined by performing six consecutive measurements on each of the QCs and Sample 4 spiked (30 ppm spiked tonic water). This was achieved by using a new aliquot of the same sample for each consecutive measurement. The calculated % RSDs (Table 3) are less than 5% for replicate measurements, and thus considered to have excellent levels of repeatability.

Levels of accuracy were assessed by comparing the average measured quinine concentrations of the two independently prepared QC samples to that of their true concentration. Table 3 illustrates the concentrations found were close to the true values, demonstrating good levels of accuracy. To further validate the accuracy of the quinine measurements and to assist in the determination of any matrix interferences, spike recovery studies were conducted. All four tonic water samples were measured in the presence and absence of a 30 ppm quinine spike. Table 4 shows the percentage recoveries of the tonic water samples, all of which with good recoveries lying between 97.7 – 102.3%.

Limit of Detection Studies

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, within a stated

Table 3. Repeatability studies (n = 6) for the analysis of quinine in tonic water using the FL6500. Concentrations shown take into account the 100-fold dilution factor.

Sample	Mean Quinine Concentration (ppm)	% RSD
QC 1 (29 ppm)	29.3 ± 1.1	3.8
QC 2 (59 ppm)	60.0 ± 1.4	2.3
Sample 4 tonic water spiked	95.5 ± 1.6	1.6

Table 3. Repeatability studies (n = 6) for the analysis of quinine in tonic water using the FL6500. Concentrations shown take into account the 100-fold dilution factor.

Tonic Water Spiked Sample	Calculated Quinine Spike Concentration (ppm)	Percentage Recovery (%)
Sample 1 tonic water spiked	29.5	101.6
Sample 2 tonic water spiked	29.3	102.3
Sample 3 tonic water spiked	30.6	98.0
Sample 4 tonic water spiked	30.7	97.7

confidence limit.[8] The limit of detection (LOD) for the determination of quinine in tonic water on the PerkinElmer FL6500 Fluorimeter was calculated from replicate analysis (n = 6) of the blank solution (0.05 M H_2SO_4) using Equation 1. The LOD, using the specified instrument settings in Table 1, was determined to be 0.009 ppm quinine in 0.05 M H_2SO_4. The limit of detection of quinine in tonic water, after considering the 100-fold dilution factor, was determined to be 0.9 ppm. These limits of detection were obtained using narrow (2.5 nm) excitation and emission slit widths. Further method optimisation using larger slit widths can provide greater sensitivity.

$$LOD = y_B + 3s_B$$

Equation 1. Calculation for the limit of detection, where y_B is the mean of the blank and s_B is the standard deviation of the blank.

Conclusion

Fluorescence spectroscopy is a very sensitive and selective analytical technique, giving it a major advantage over absorption spectroscopy when used in the detection and measurement of trace amounts of organic compounds. The PerkinElmer FL6500 Fluorescence Spectrometer with Singe Cell accessory provides an accurate, simple and repeatable method for the determination of quinine in tonic water samples. Owing to the dark correction features in Spectrum FL software, samples can be analyzed with the sampling compartment lid open or closed. The ease of use of the FL6500 and Spectrum FL software, makes it highly suitable for fluorescence applications in academia and routine fluorescence analysis in QA/QC labs. The Spectrum FL Enhanced Security (ES) software package also provides additional security and data integrity features for achieving compliance with 21 CFR Part 11.

References

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