Quantify Quinine in Beverages Using the Agilent Cary Eclipse Spectrofluorometer and a Fiber Optic Dip Probe

Simplify Analysis, Improve Workflow, and Save Money

Application Note

Food Testing and Agriculture

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Introduction

Every effective and efficient manufacturing process is based on well-designed and methodical quality control protocols that simultaneously maximize productivity and ensure product quality. Production line managers cannot waste time once a defect has been identified. In a competitive market, any solution that minimizes downtime and enables a rapid and successful return to production is an important advantage.

An accurate, reliable, and seamless screening process is essential to quickly identify production anomalies. Fiber optic-based analytical solutions have been embraced globally as an option wherever rapid time measurements are essential.

One area where fiber optic-coupled solutions have been underused, however, is in routine fluorescence spectrophotometry, because it has been difficult to implement room-light immunity to the level that is required for accurate and reproducible measurements. Fiber optic fluorescence analytical solutions reduce measurement time, provide accurate and robust data from samples in countless applications, and save money by streamlining the analytical process and reducing the overall cost of ownership. The Agilent Cary Eclipse fluorescence spectrophotometer provides a unique solution to this issue.

Fluorescence spectrophotometry is up to 1,000 times more sensitive than other optical spectroscopic methods. It is also highly selective, easy to use, and ubiquitous in a broad range of QA/QC applications. The Cary Eclipse fluorescence spectrophotometer is unique because it has been designed and engineered with complete room-light immunity in mind. The instrument ensures that the significant advantages of measurements made using fiber optics (no need for cuvettes, faster analysis times, and reduced potential for contamination) are not compromised at the expense of the quality of the results. This application note illustrates the unique advantages of the Cary Eclipse using an example from the food and beverages industry.

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The quality of food and drink has never been under such intense scrutiny, and all areas of food production call for high standards in quality and process control. These demands require sensitive and rapid analytical technologies. Fluorescence spectrophotometry is proving critical in many aspects of food production because it can deliver detailed and real-time observations of key substances in many food systems, including proteins, vitamins, secondary metabolites, pigments, toxins, and flavoring compounds (Table 1) [1,2,3].

Table 1. Spectroscopic data of some key substances often found in food products.

Key substance	Excitation λ_{max} (nm)	Emission λ_{max} (nm)
Quinine	250 (350)	450
Tyrosine	278	302
Tryptophan	276 (230)	355
Vitamin A (retinol)	328	484
Vitamin B_2 (riboflavin)	268 (272, 444)	528
Vitamin B_6 (pyridoxine)	326	396
Vitamin E (α-tocopherol)	298	323
Folic acid	355 (285)	440
NADH	338	465
ATP	292	392
Chlorophyll a	428	663
Hematoporphyrin	394	613
Ponceau 4R (food dye)	376	621
Amaranth (food dye)	370	643
Tartrazine (food dye)	315	565
Sunset yellow (food dye)	348	592
Brilliant blue (food dye)	350	456

Experimental

A rapid and accurate method of quantifying quinine in liquid samples was sought. Speedy yet reliable measurements present a complex analytical challenge for any manufacturer. Therefore, the brief was to design an experimental protocol that would allow a "real-time" decision to be made should the quality of quinine-containing solutions fall below strict customer specifications.

Analytical approach

Samples were analyzed using the Cary Eclipse fluorescence spectrophotometer. Comprehensive sample analyses were made using the traditional method of filling cuvettes with a standard or a sample, and analyzing the contents. This is followed by emptying and cleaning the cuvette thoroughly before refilling. Samples were also analyzed *in situ* using the remote measurement capabilities that are uniquely offered by the Cary Eclipse coupled with a fiber optic probe. For these measurements, a fiber optic liquid probe tip with black quartz base (path length 10 mm) was used to directly measure every sample in its own container. There was no need to fill, empty, and clean any cuvettes. By removing the need for this tedious process, analysis time was reduced to two-thirds of that required by traditional measurements. All data were collected and processed using the Scan software application in WinFLR.

Samples

Quinine is an alkaloid naturally found in the bark of cinchona trees. It shows strong bioactivity, and was for many years the therapeutic of choice for the treatment of malaria. Today, it is most frequently found in tonic water where its bitter taste gives this beverage its characteristic flavor. Quinine is also fluorescent and, because it is readily available, is ideal to effectively illustrate the reproducibility and sensitivity of the Cary Eclipse fiber optics-based fluorescence measurements.

Reagents

Quinine sulphate powder, ≥98.0%, was purchased from Sigma-Aldrich, Corp. The solvent was 0.05 M H_2SO_4, and the samples consisted of four brands of bottled tonic water bought from a local supermarket.

Results and Discussion

Creating calibration curves

Five different standard solutions, with quinine concentrations ranging from 1.0 to 0.10 mg/L, were prepared using quinine sulphate and 0.05 M H_2SO_4. Initially, we dissolved 6.0 mg of quinine sulfate in 500 mL 0.05 M H_2SO_4, then diluted 25 mL of this solution with 225 mL 0.05 M H_2SO_4 to give a stock solution of 3.06×10^{-6} mol/L. From this stock solution, 5 x diluted calibration standards with concentrations reaching from 3.06×10^{-6} mol/L (1 ppm) to 3.06×10^{-7} mol/L (0.1 ppm) were prepared in standard PET reaction glass vials (diameter 16 mm).

Samples of each concentration were transferred into standard 10 × 10 mm cuvettes and measured to generate calibration curves. The same standards were also measured by dipping the fiber optic probe into each reaction glass vial. Again, we calculated calibration curves for the relationships between standard concentration and fluorescence intensity

Analyzing unknowns

Four brands of bottled tonic water bought from a local supermarket were analyzed as unknowns. A 5 mL sample of every tonic water was shaken to remove the dissolved carbon dioxide, then diluted in 500 mL 0.05 M H_2SO_4 (dilution 1:100). As per the calibration standards, aliquots of each unknown were transferred into separate 10 × 10 cuvettes and measured. The diluted tonic water samples in each cuvette were then measured using the fiber optic probe.

Samples in the production environment

These analog experiments, where a calibration curve is generated and then various unknowns are measured, accurately replicate those found in regulatory laboratories. The most common method for analyzing solutions such as tonic water involves pouring a sample into a cuvette (a well-known source of contamination) and measuring the contents. The cuvette is then emptied (a source of waste), cleaned, and dried (another well-known source of contamination) before the next measurement can be made. Using the fiber optic probe simply involves dipping the probe in the sample and taking a measurement. The probe tip is wiped clean with a cloth, and is then ready for the next sample.

Fluorescence spectrophotometry is the ideal tool for the nondestructive quantification of intrinsically fluorescing chemical species in any chemical environment. Spectra collected using the Cary Eclipse show that quinine in H_2SO_4 has a characteristic shape and an intensity that is directly proportional to the concentration of quinine in the sample. Figure 2 shows the normalized emission spectra of a bottled tonic water sample (tonic water 1) measured with the sample compartment of the spectrophotometer open and closed. It also shows the same sample measured using a fiber optic dip probe attached to the Cary Eclipse. Thanks to the unique room-light immunity of the Cary Eclipse, the spectra collected in each measurement are identical. Using the relationships between fluorescence intensity of the quinine band and the known quinine concentration of the standard, we quickly, easily, and accurately determined the concentration of quinine in the tonic waters. Remember that measured concentrations were multiplied by 100 to yield the actual concentration because the samples were diluted 1:100.

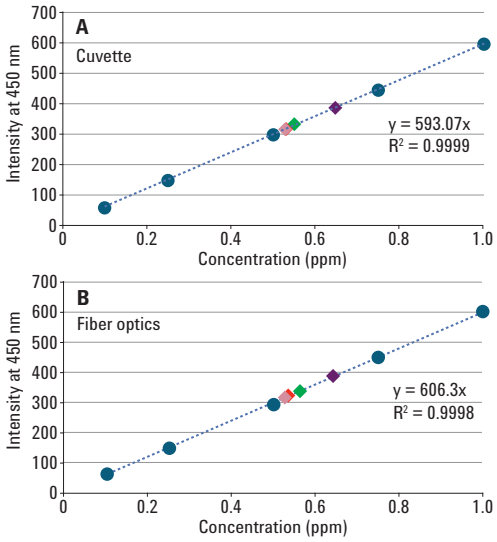

Figure 1. Calibration curves for quinine standards (filled circles) and four different tonic waters (filled diamonds). Analyses of the standard solutions and the unknowns were conducted using fiber optics and glass cuvettes. Data from both methods are identical.

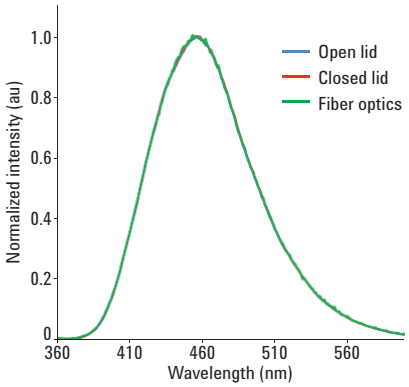

Figure 2. Normalized emission spectra of a bottled tonic water sample (tonic water 1) measured using an Agilent Cary Eclipse with the sample compartment open, closed, and using the fiber optic accessory (excitation at 350 nm, averaging time one second).

Several important observations can be made from our measurements:

- Selective quantification of quinine in aqueous samples is easy with the Cary Eclipse.
- Appropriate molecular species can be accurately and reproducibly quantified using traditional cuvette-based measurements or by embracing the unique capacity of the Cary Eclipse to use remote sensing, fiber optic probes.
- The quinine concentration in commercially available bottled tonic water differs between brands.
- Commercially available tonic waters have quinine contents between 50 and 60 ppm (Table 2), beneath the maximum quinine concentration of 83 ppm permitted by the U.S. Food and Drug Administration [4].

Table 2. Quinine concentrations in four different tonic water brands determined using an Agilent Cary Eclipse with a standard glass cuvette and the fiber optic accessory.

	Quinine content (ppm)	
Sample	**Standard cuvette***	**Fiber optic***
Tonic water 1	65 ± 0.2	64 ± 0.5
Tonic water 2	55 ± 0.2	56 ± 0.4
Tonic water 3	53 ± 0.3	53 ± 0.5
Tonic water 4	53 ± 0.2	53 ± 0.7

*Average of 10 measurements

Conclusions

The Agilent Cary Eclipse, equipped with the fiber optic accessory, delivers fast, simple, and accurate measurement of fluorescent substances in drink and food products. We have shown that measurements using the fiber optic accessory are as accurate as those performed using standard quartz glass cuvettes. In addition, the workflow is significantly faster and removes steps that are known to introduce measurement errors. Not only does the fiber optic accessory allow extremely small samples to be accurately and reproducibly measured, but it also eliminates the need for cuvettes. These features make the Cary Eclipse and fiber optics the ideal instrument for applications in routine fluorescence analysis in QA/QC labs.

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