APPLICATION NOTE

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UV-Vis chemometric analysis of azo dyes using NanoDrop QC Software

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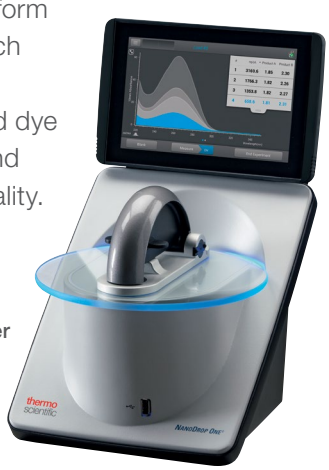
Key words
NanoDrop QC, chemometrics, UV-Vis, microvolume spectrophotometer, spectral analysis, dyes, TQ Analyst, dye mixtures, partial least squares (PLS), multicomponent analysis, UV-Vis chemometric analysis, azo dyes, dilution, high absorbance

Abstract
The new Thermo Scientific™ NanoDrop QC™ Software for the Thermo Scientific™ NanoDrop™ One^c Microvolume UV-Vis Spectrophotometer allows scientists to perform chemometric analysis of high absorbance chemical samples in real-time without the need to dilute. In this note, we demonstrate a step-by-step approach to create and validate a chemometric method that determines individual dye concentrations in a complex mixture. We show how NanoDrop QC Software can be used to obtain quantitative information in samples that have multiple components with highly overlapping UV-Vis spectra. The method we created determines the individual concentrations of tartrazine and sunset yellow in mixtures containing different ratios of each component and determines the percentage of each dye in the mixture. NanoDrop QC Software for the NanoDrop One^c Spectrophotometer extends the patented microvolume measurement platform to a wide range of industries such as petrochemical companies, polymer manufactures, and food dye producers, which need a fast and accurate way to test sample quality.



Thermo Scientific NanoDrop One^c Microvolume UV-Vis Spectrophotometer

Introduction

Ultraviolet-visible (UV-Vis) spectroscopy is an analytical technique widely used to determine quantitative information about chemical species because it is inexpensive, fast, and accurate. However, its accuracy will depend on how the spectral data is analyzed. For pure chemical samples, where the wavelength-dependent extinction coefficient is known, Beer's Law can be used to calculate the concentration. A known limitation of Beer's Law is that it can only produce accurate results in samples where there are no other chemical species with overlapping absorbance at the analytical wavelength.[1]

Chemometrics has been widely used to analyze UV-Vis data from complex chemical samples (i.e., mixtures). Chemometrics provides relevant chemical system information by analyzing measured chemical data[2] and provides a powerful approach to determining the concentration of chemical species that have overlapping spectra. Chemometrics is the use of multivariate mathematical models and various statistical techniques to determine quantitative concentration information of many components simultaneously.[3] Unfortunately, the sophistication of the multivariate calibration models used in chemometrics has limited its use to individuals with deep knowledge of the field. Thus, most data analysis is done by an experienced chemometrics expert after the data have been collected.

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The NanoDrop QC Software combines powerful chemometric analysis with the NanoDrop One^{c}'s microvolume measurement platform. This measurement platform utilizes a revolutionary sample retention technology, which retains 1 to 2 µL samples in place via surface tension between two fiber optic cables (Figure 1).[4] The measurement platform also uses multiple pathlengths (1.0 mm, 0.2 mm, 0.1 mm, 0.05 mm, and 0.03 mm) that change in real-time while measuring the sample, thus resulting in a wide dynamic range that expands from 0.04 to 550 absorbance units (10 mm equivalent absorbance units). These characteristics make the instrument's platform ideally suited for a wide variety of industrial applications, such as polymer QA/QC, petrochemical analysis, industrial dye analysis, and other material science applications.

To show the utility of the NanoDrop QC Software in performing real-time chemometric analysis, we mixed two common dyes (tartrazine and sunset yellow) and built a multivariate calibration model to determine the concentration of each component and the % composition of each component in the mixture. To demonstrate the applicability of this method, we chose dyes with UV-Vis absorption spectra that highly overlapped each other. We also describe the creation and validation of the chemometric method.

Materials and methods

To demonstrate the power of a chemometric analysis run on our microvolume UV-Vis platform, we developed an experimental system with mixtures of two water-soluble azo dyes, tartrazine and sunset yellow. These two dyes were chosen for the experimental system because large regions of their spectra overlap. Tartrazine has two prominent peaks located within the UV-Vis spectrum (259 nm, 425 nm), whereas sunset yellow has three peaks within the UV-Vis region (238 nm, 315 nm, 476 nm) (Figure 2).

The concentrations of each dye chosen for these experiments were high enough so that it would be impossible to quantitatively determine the concentration of each dye in a mixture by using traditional spectroscopy methods (e.g., Beer's Law and measurements in cuvettes).

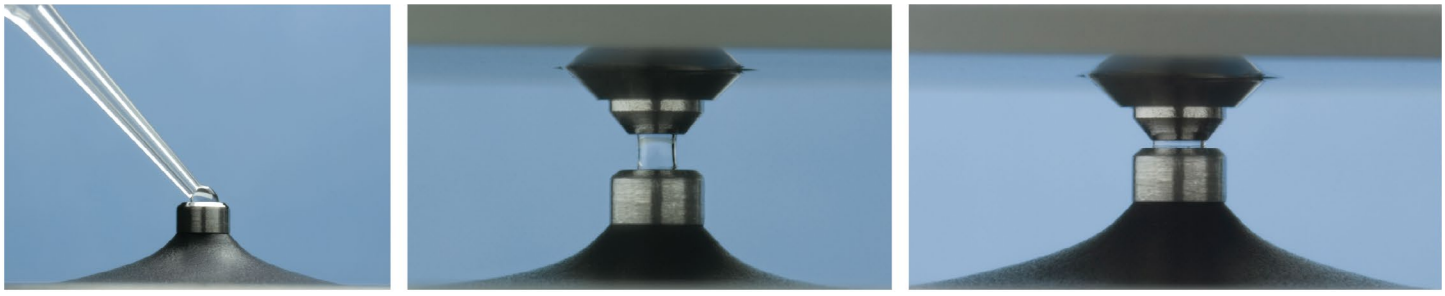

Figure 1: NanoDrop Microvolume Sampling platform
Left: Loading of a 1 µL sample on the measurement pedestal
Middle: Sample measurement at 1 mm pathlength
Right: Sample measurement at 0.2 mm

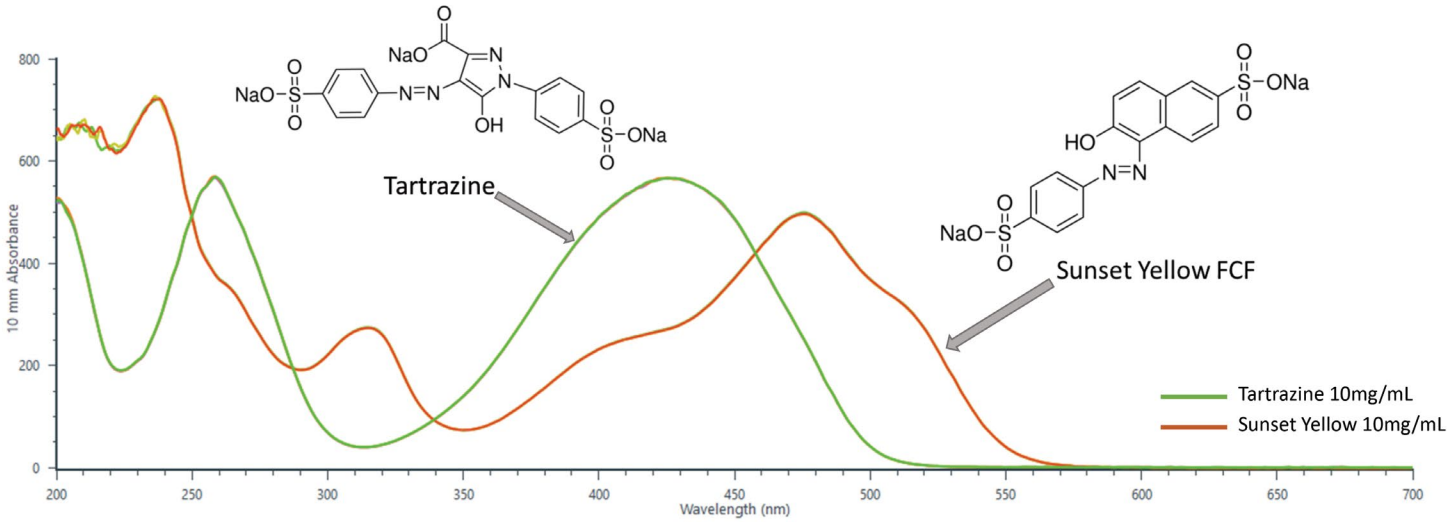

Figure 2: Full UV-Vis spectra of pure tartrazine and sunset yellow (200 nm – 700 nm). Spectra was collected with the UV-Vis module of the NanoDrop QC Software and was baseline corrected at 800 nm. Solutions of each dye were prepared at 10 mg/mL and 2 µL aliquots were measured on the instrument.

Experimental flow chart

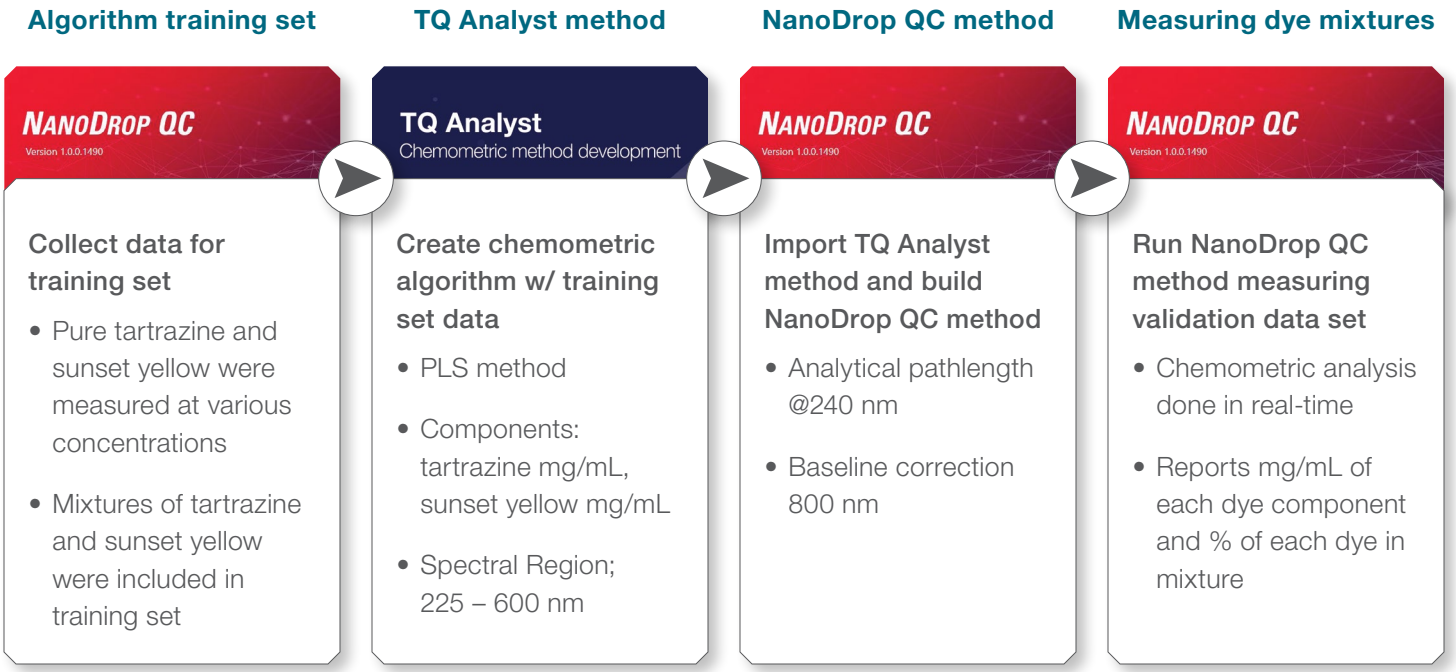

Figure 3: Experimental steps followed to create and test a NanoDrop QC chemometric method to determine tartrazine and sunset yellow dye concentration in a mixture.

Algorithm training set

Table 1 describes the training set samples that were prepared to create the chemometric method and to determine tartrazine and sunset yellow dye concentrations in a mixture. The following stocks were made by serially diluting 100 mg/mL dye solutions in ddH_2O: 10 mg/mL, 2.5 mg/mL, and 0.5 mg/mL. The appropriate volumes of each stock were pipetted to yield 100 µL of dye mixtures. Then 2 µL aliquots were measured with NanoDrop QC Software using the UV-Vis application. Baseline correction was performed at 800 nm and pathlength control was set to an analytical wavelength at 240 nm. Three replicates were run for each sample.

TQ Analyst method

The training set data was imported into Thermo Scientific™ TQ Analyst™ Software and a partial least squares (PLS) method was developed. The spectral region from 225 nm to 600 nm with a baseline correction at 800 nm was used to create the calibration. There was no other spectral processing performed on the data. The two components used were tartrazine concentration (mg/mL) and sunset yellow concentration (mg/mL). Two composite calculations were also created to calculate the % composition, i.e., % tartrazine and % sunset yellow. The method uses 4 factors to determine the sunset yellow concentration and 3 factors to determine the tartrazine concentration.

Mixture training set			
Training set sample #	**Description**	**Tartrazine (mg/mL)**	**Sunset yellow (mg/mL)**
1	Mixture	7.46	2.54
2	Mixture	0.38	9.62
3	Mixture	5.92	4.08
4	Mixture	2.43	7.57
5	Mixture	8.00	2.00
6	Mixture	3.74	6.26
7	Mixture	5.22	4.78
8	Mixture	2.07	0.43
9	Mixture	0.42	2.08
10	Mixture	1.61	0.89
11	Mixture	1.88	0.62
12	Mixture	1.03	1.47
13	Mixture	1.54	0.96
14	Mixture	0.09	0.41
15	Pure	0.00	0.50
16	Mixture	0.19	0.31
17	Mixture	0.15	0.35
18	Mixture	0.36	0.14
19	Mixture	0.22	0.28

Table 1: Samples created for the chemometric algorithm training set. The training data set consisted of randomly determined mixtures of tartrazine and sunset yellow. The concentration range spanned from 0 mg/mL to 10 mg/mL.

NanoDrop QC method

To create a NanoDrop QC method, we imported the TQ method described above into the NanoDrop QC Software. The analytical wavelength of 240 nm was selected to optimize the pathlength chosen for the best measurement result.

Method validation

Measuring dye mixtures

Table 2 describes the validation samples made for the validation of the method created. The stocks in Table 2 were made by serially diluting 100 mg/mL dye solutions in ddH_2O to obtain 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.0 mg/mL, and 0.5 mg/mL stocks. The appropriate volumes of each stock were pipetted to yield 100 µL of either pure dye samples or dye mixtures. Then 2 µL aliquots were measured on the NanoDrop One^c instrument with the chemometric method application. Pathlength control was set to the analytical wavelength of 240 nm. Three replicates were run for each sample. To check the accuracy of the chemometric method, we compared the expected (Table 2) and measured concentrations of each dye in the mixtures.

Validation samples			
Validation sample #	**Description**	**Tartrazine (mg/mL)**	**Sunset yellow (mg/mL)**
1	Mixture	4.19	5.81
2	Mixture	1.79	8.21
3	Mixture	5.17	4.83
4	Pure sunset yellow	0.00	5.00
5	Mixture	1.34	3.66
6	Mixture	4.59	0.41
7	Mixture	0.32	2.18
8	Mixture	1.61	0.89
9	Mixture	1.91	0.59
10	Mixture	0.39	0.61
11	Pure tartrazine	1.00	0.00
12	Mixture	0.30	0.20
13	Mixture	0.25	0.25
14	Mixture	0.31	0.19

Table 2: Samples created to validate the chemometric method described above. Samples spanned the concentration range from 0 mg/mL to 8.21 mg/mL.

Results

Nanodrop QC Software allowed us to run samples and perform a chemometric analysis in real-time. Figure 4 shows how the data is presented by the software.

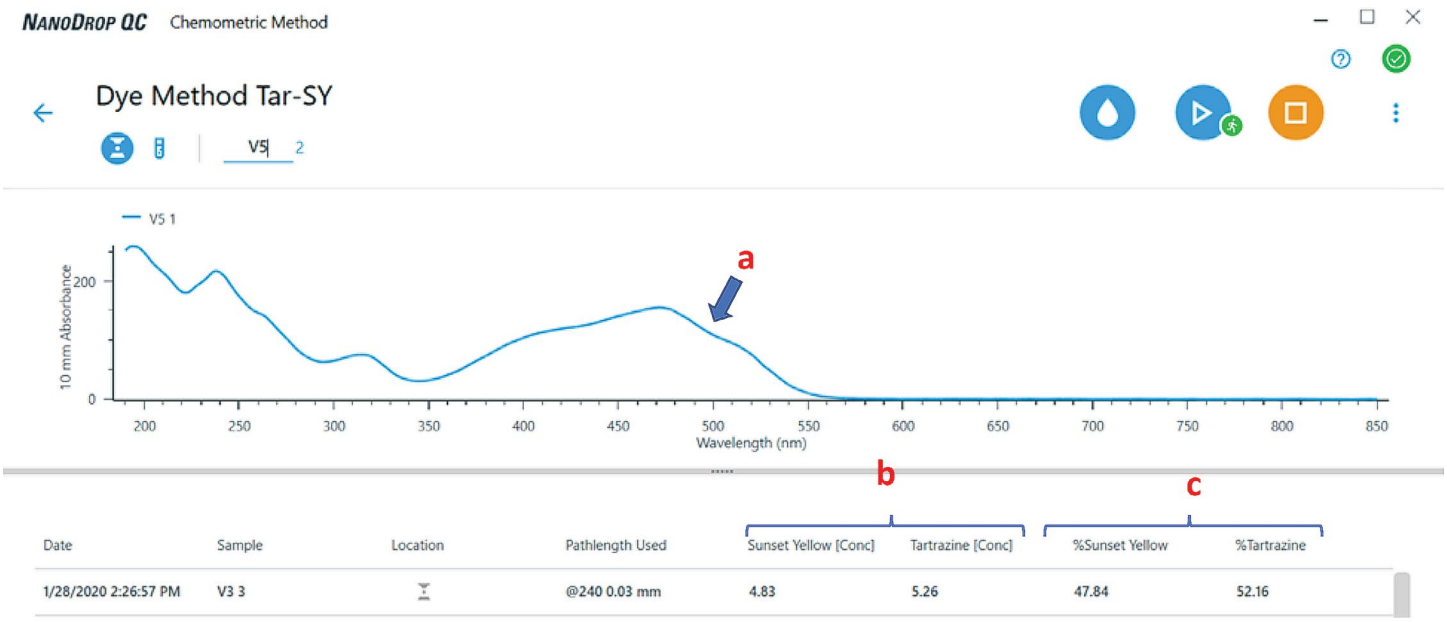

Figure 4: View of the chemometric method application contained within the NanoDrop QC Software. Note that the component (dye concentration) and composite results are reported directly on the screen in real-time. There is no need to perform any post-run data processing. For each mixture, the software displays (a) the spectrum of the mixture and (b) the concentration and (c) % composition of each dye in that mixture.

The concentrations obtained for the 14 dye mixtures correspond nicely to the expected dye concentrations (Figures 5 & 6). The validation samples spanned a wide concentration range (0 mg/mL to 8.21 mg/mL), thus allowing us to evaluate the full range of dye concentrations used in the training set. In both cases (Figures 5 & 6), the chemometric prediction for the highest dye concentrations showed the largest discrepancies. When comparing the tartrazine results to the sunset yellow results, sunset yellow had more error in its prediction. However, the difference seen between expected concentration and observed concentration still only ranged from 0.04 mg/mL – 0.28 mg/mL. Discrepancies discussed above emphasized the importance of validating chemometric methods by testing the performance of the algorithms with independently generated samples.

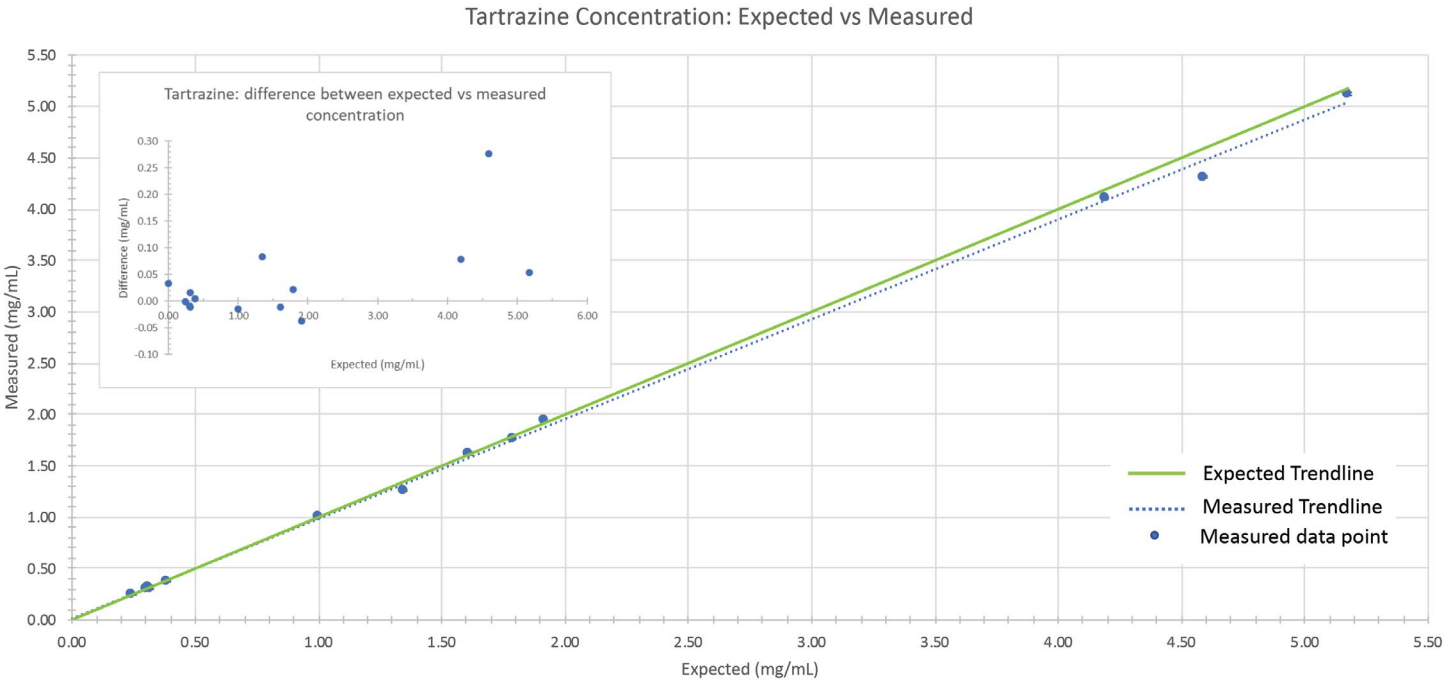

Figure 5: Comparison between expected vs. measured tartrazine concentration of the validation sample set. The green line represents the trendline when expected concentrations are plotted against themselves (i.e., measured values perfectly match expected values). The dotted blue line is the observed trendline when the measured concentrations are plotted against the expected concentrations.

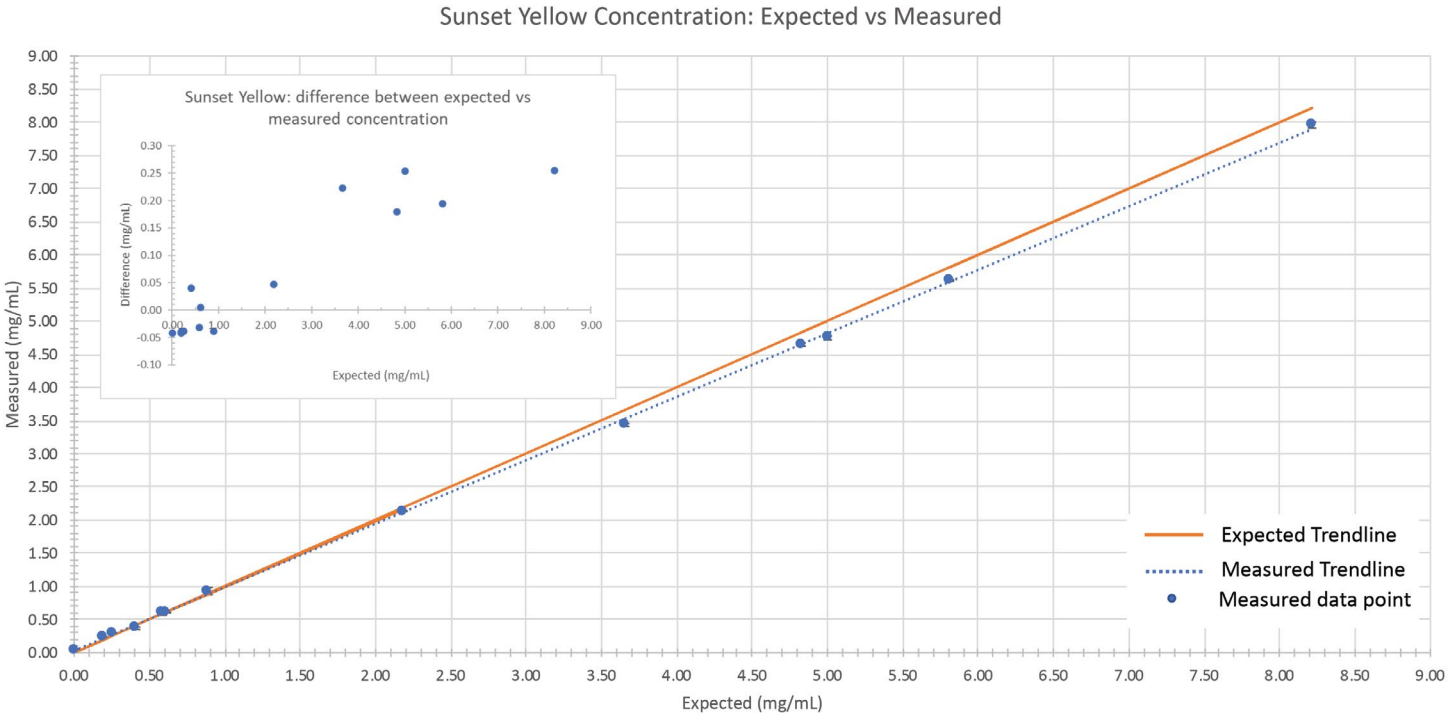

Figure 6: Comparison between expected vs. measured sunset yellow concentration of the validation sample set. The orange line represents the trendline when expected concentrations are plotted against themselves (i.e., measured values perfectly match expected values). The dotted blue line is the observed trendline when the measured concentrations are plotted against the expected concentrations.

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Conclusion

NanoDrop QC Software coupled with the NanoDrop One^{c} microvolume sampling platform offers several advantages to scientists, including the ability to:

- Measure highly absorbing samples (>500 A) without the use of specialized short-pathlength cuvettes, specialized flowcells, or performing laborious dilutions
- Provide full UV-Vis spectral data in a 10-second measurement (1,300 data points)
- Run chemometric analysis in real-time to streamline data processing steps

We were able to create and perform chemometric analysis on various mixtures of highly concentrated dyes. The wide applicability of UV-Vis measurements paired with chemometric analysis can become a very attractive solution for a wide range of applications including petrochemical chemical analysis, drug purity,[5] polymer manufacturing, food dye applications, and many more applications where chemometric analysis is required.

References

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