Application note | AN1218

UV-Vis spectroscopy

Use of UV-Visible absorption measurements for Pt-Co color analysis and yellowness index calculations

Introduction

In wastewater and drinking water analyses, there are a variety of tests which need to be performed to assess the quality of the water.[1,2] While many of these methods detect the presence of specific substances, some are related to the appearance of the water itself. For example, the degradation of plant matter can produce an appearance of a yellow color to the sampled water.[2] While this change in water color does not inherently indicate a health concern, a yellow hue to a water source, especially drinking water, is perceived negatively by the general public.[1]

The color of a substance arises from the reflection/scattering of visible light off a surface. As this relates to the visible region of the electromagnetic spectrum, UV-Visible spectroscopy can be used to assess the color of a substance. There are a variety of established methods which use the UV-Visible spectrum to determine the color of an object. One frequently used method for assessment of water and wastewater quality is known as the platinum-cobalt method, or Pt-Co color scale.

Pt-Co, Hazen, and APHA color are three different terms referring to the same color analysis procedure often used to assess the yellowness of water samples. In this analysis a mixture of cobalt chloride and potassium hexachloroplatinate(IV) is prepared and subsequently diluted by varying amounts to form an array of yellow to almost colorless standards, as described in ASTM D1209 and ISO 6271:2015.[3,4]

In some methods, these standards are visually compared to the sample, and samples are then given a Pt-Co color value equivalent to the concentration of the standard which most closely matches the sample's yellowness. Note that as the standards used to establish these scales are solution-phase, this method is only appropriate for solution-phase samples. By its nature, this method is subjective and can lead to differences in reported Pt-Co color values, for the same concentrations, from person to person.

To provide a method of comparison which is independent of the user, UV-Visible absorption measurements can be used instead. Through this methodology, the spectra for the Pt-Co color standards are used to provide coordinates in the tristimulus color space, as described in ASTM E308.[5] By the nature of this calculation, the tristimulus color values provide a uniform coordinate system to assess a sample's color. These values can then be used to determine the yellowness index, as outlined in ASTM D313.[6] A calibration curve relating the calculated yellowness index and Pt-Co color values is then established.[3]

Note that the Pt-Co scale is dependent on the spectra of Pt-Co standards only. As many substances will have different absorption spectra, the comparison against Pt-Co color standards will not be a perfect analysis of yellowness. For solutions which appear to be more yellow/red or yellow/blue than the Pt-Co standards, the calculated Pt-Co value may be skewed from the expected Pt-Co value.

Alternatively, the yellowness index can be used in place of Pt-Co to provide a value for how yellow a given sample appears. This analysis is not dependent on a specific standard substance, unlike the Pt-Co color scale. Instead, comparisons between the yellowness index values of different solutions can be made to an established yellowness index value, and thus ensure a sample meets the criteria for how yellow it appears. Because the spectrophotometric determination of Pt-Co uses the yellowness index calculation, the reported color value will be less biased than a value ascertained through visual inspection.

Herein, the Pt-Co and yellowness index color calculations were used to analyze several yellow solutions. Theses samples include Pt-Co standards and food dye, as well as dirt and grass solutions, to mimic possible water sources found outside of a lab environment. Spectra were collected using the Thermo Scientific™ Evolution™ One UV-Visible Spectrophotometer. Calculations were carried out using the Thermo Scientific™ Insight™ Pro Software, allowing for a quick analysis without needing manual data analysis.

Experimental

Sample preparation

The procedure outlined within ASTM D1209[3] was adapted to provide a smaller stock solution of the Pt-Co standard. Briefly, 0.1246 g of potassium hexachloroplatinate(IV) and 0.10 g of cobalt chloride hexahydrate were dissolved in 100 mL of 1.2 M HCl. The solution was sonicated using a bath sonicator to ensure the solids were completely dispersed in solution. The concentration of Pt in the stock solution was 500 ppm. As such, this solution is referred to as the 500 Pt-Co color standard. Subsequent Pt-Co color standards were made by diluting the required amount of Pt-Co stock solution with DI water (Table 1).

Pt-Co color standard	Volume 500 Pt-Co color standard (mL)	Volume DI water (mL)
350	10.5	4.50
250	12.5	12.5
200	10.0	15.0
150	7.50	17.5
100	5.00	20.0
70	3.50	21.5
25	1.25	23.75
15	0.75	24.25
10	0.30	14.70
5	0.25	24.75
2	0.06	14.94

Table 1: Preparation of Pt-Co color standards.

300 µL of a commercially available yellow food dye solution (YFD) was added to 50 mL of deionized water to prepare a stock solution. As the concentration of the dye was not disclosed on the packaging, an exact concentration of food dye measured in the experiments described here could not be provided. Instead, the solutions are reported percent food dye (v/v) in DI water. Three sample solutions were made of YFD with concentrations of 0.0012%, 0.0026% and 0.0077%.

Two separate solutions were prepared as examples of real environmental samples. First, a dirt solution was prepared by adding 0.7415 g of indoor plant soil to 25 mL of DI water. The solution was mixed and allowed to sit for 5 days. A portion of the solution was then filtered using a 0.22 µm PVDF syringe filter to remove any particulates. The solution was then diluted by a factor of two for UV-Visible analysis. Second, a solution with three blades of grass in 50 mL of DI water was prepared and allowed to sit for 8 days. The stock grass solution was used as prepared with no further dilution or filtration.

Instrumentation

For all UV-Visible absorption measurements, samples were held in a 1.0 cm-pathlength quartz cuvette. Spectra were collected from 380 nm to 780 nm using a 1.0 nm step size. The integration time was set to 0.5 s and a 1.0 nm bandwidth was used. DI water was used to collect the blank/background. All color analyses (i.e., Pt-Co color values, yellowness index, etc.) were carried out using the built-in color calculations within the Insight Pro software. Each sample was prepared and measured in triplicate with the average color values reported herein.

Results/discussion

Pt-Co standards

Figure 1a includes the UV-Visible spectra for Pt-Co standards of varying concentrations, prepared as described previously. The spectra include a peak at 458 nm as well as an absorption band edge extending into the UV region. Within the concentration range measured, the absorbance at 458 nm adheres well to a linear function, as shown in Figure 1b. This linearity indicates the concentration range measured falls within the dynamic range and could be used for quantification purposes.

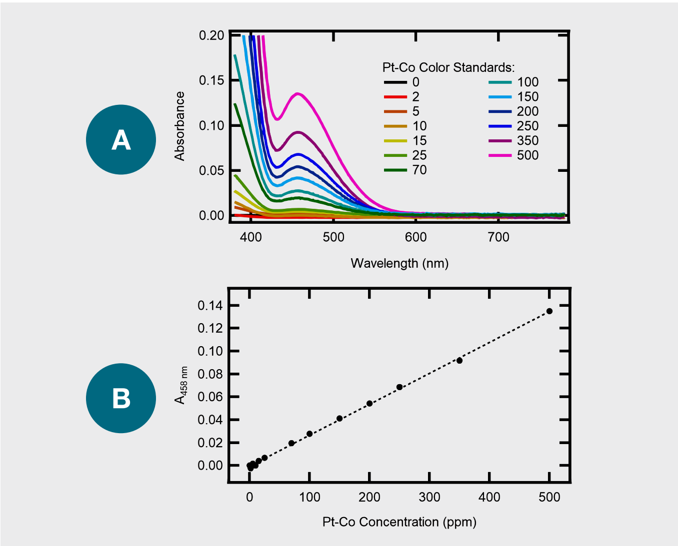

Figure 1: (a) UV-Vis absorption spectra of various Pt-Co color analysis standards. (b) Average absorbance measured at 458 nm as a function of Pt-Co color value.

These standards are used to form the basis of the Pt-Co color analyses, where the concentration of Pt-Co in ppm is used to denote the color value of a given sample. However, as not all yellow samples will have a peak maximum at 458 nm, an alternative method must be employed to represent the color, or “yellowness”, of the sample. Instead, the Pt-Co color value is calculated based on the full spectrum rather than a single absorbance, as described previously. This involves the calculation of the tristimulus color values (X, Y and Z), which in turn are used to calculate the yellowness index of the substance. While the calculations used to determine X, Y and Z values are described elsewhere,[5,7] the yellowness index can be calculated according to Equation 1,

$$YI = \frac{100(C_x X - C_y Y)}{Z} \quad (1)$$

where X, Y and Z are the tristimulus color values and C_x and C_y are yellowness index coefficients outlined in ASTM E313 and are specific to the assumed illuminant and observer angle.[6] For the purposes of this calibration, the tristimulus values and yellowness index coefficients specific to the illuminant C and 2° observer angle are used. A calibration curve is then constructed relating the Pt-Co value or each standard with the calculated yellowness index value. This calibration is included in the Insight Pro software.

Table 2 includes the Pt-Co color values calculated for select Pt-Co color standards using the Insight Pro software. Additionally, the yellowness index (illuminant C and 2° observer) was calculated for each Pt-Co color standard (Table 2). The calculated Pt-Co color values, qualitatively, are closely matched to the expected Pt-Co standard values. For a more quantitative comparison, the percent difference between the anticipated Pt-Co color value and the calculated value was determined for each standard and also included in Table 2. As shown, the percent difference is minimal for Pt-Co color standards 70 and above; however, for Pt-Co standards 25 and below, the percent difference is above 5%. To assess colors within this range, it is better to use a longer path length so as to overcome these issues.

Pt-Co color standard	Pt-Co color value		Yellowness index (illuminant C, 2° observer)*
	Value	Percent difference (%)	
500	498 ± 1	0.4	24.2 ± 0.04
350	348 ± 2	0.6	17.4 ± 0.1
250	252 ± 1	0.8	12.82 ± 0.05
200	201.3 ± 0.7	0.7	10.32 ± 0.03
150	150.9 ± 0.5	0.6	7.80 ± 0.02
100	101.9 ± 0.1	1.9	5.29 ± 0.01
70	72.1 ± 0.3	3.0	3.73 ± 0.02
25	26.9 ± 0.2	7.3	1.32 ± 0.01
15	16.9 ± 0.3	11.9	0.78 ± 0.02
10	10.80 ± 0.06	7.7	0.46 ± 0.01
5	7.4 ± 0.1	38.7	0.32 ± 0.08
2	2.7 ± 0.4	29.8	0.03 ± 0.01
0	2.3 ± 0.1	200	-0.01 ± 0.01

* Standard deviation calculated using the raw data.

Table 2: Pt-Co color value and yellowness index values for prepared Pt-Co standard solutions. The yellowness index was determined using the CIE L*a*b* values calculated using Illuminant C and a 2° observer. Percent difference calculations were carried out using the average calculated Pt-Co color value and the Pt-Co standard value.

Figure 2: Color calculation function in the Insight Pro software.

Yellow food dye

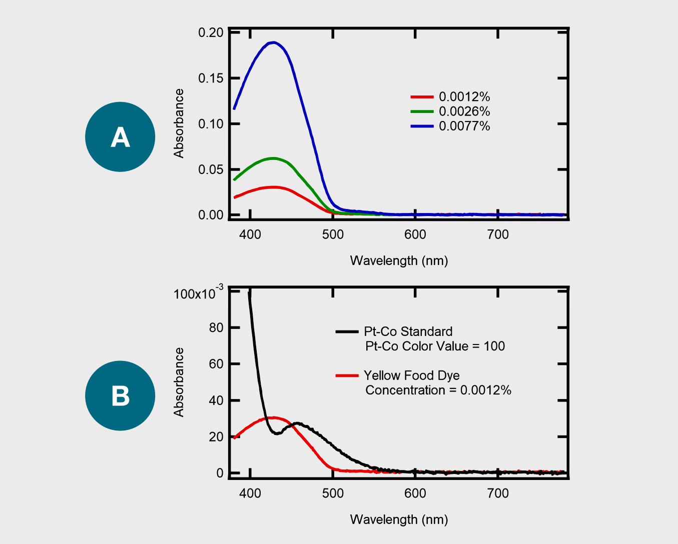

Figure 3: (a) UV-Visible absorption spectra of yellow food dye samples of varying concentration. (b) Comparison of UV-Visible absorption spectra of 0.0012% yellow food dye (red) and Pt-Co 100 color standard.

UV-Visible spectra of a commercially available yellow food dye, in prepared solutions of varying concentrations, were collected (Figure 3a). The yellow food dye analyzed herein has a maximum absorbance at 428 nm. Note that the spectrum for yellow food dye does not match the spectrum for the Pt-Co standard as shown in Figure 3b. Though the spectra do not match, the Pt-Co color values can be used instead to assess the color. Table 3 includes the Pt-Co color values for each food dye sample as well as the calculated yellowness index values. As shown in Table 3, the 0.0012% yellow food dye sample has a Pt-Co color value close to the 100 Pt-Co standard, while the color value for the 0.0026% yellow food dye sample was closest to the 200 Pt-Co standard. The highest concentration yellow food dye sample was too concentrated and therefore resulted in a Pt-Co value outside the limits of the measurements (>500).

Yellow food dye concentration (% (v/v))	Pt-Co color value	Yellowness index (illuminant c, 2° observer)*
7.7 x 10^{-3}	>500	26.2 ± 0.3
2.6 x 10^{-3}	186 ± 3	9.6 ± 0.1
1.2 x 10^{-3}	92.1 ± 0.7	4.73 ± 0.03

* Standard deviation calculated using the raw data.

Table 3: Pt-Co color values and yellowness index (illuminant C, 2° Observer) determined for yellow food dye samples of varying concentration.

According to ASTM D1209,[3] one method for assessing the Pt-Co color value is through visual inspection. In this method, a set of Pt-Co color standards are compared against the prepared samples. These standards and samples are held in a similar vessel to allow for a better comparison. Figure 4 includes images of various Pt-Co standards compared with the 0.0012% and 0.0077% yellow food dye samples. For the 0.0077% YFD samples, it is not difficult to visualize that the sample is more concentrated than both the 250 and 500 Pt-Co standards. However, for the 0.0012% YFD, it is difficult to tell how close the sample is to the 70, 100 and 150 Pt-Co standards by eye, demonstrating how difficult and inherently biased the visual assessment can be. Alternatively, using UV-Visible spectroscopy helps avoid these biases, providing an impartial assessment of color, as shown by the results in Table 3.

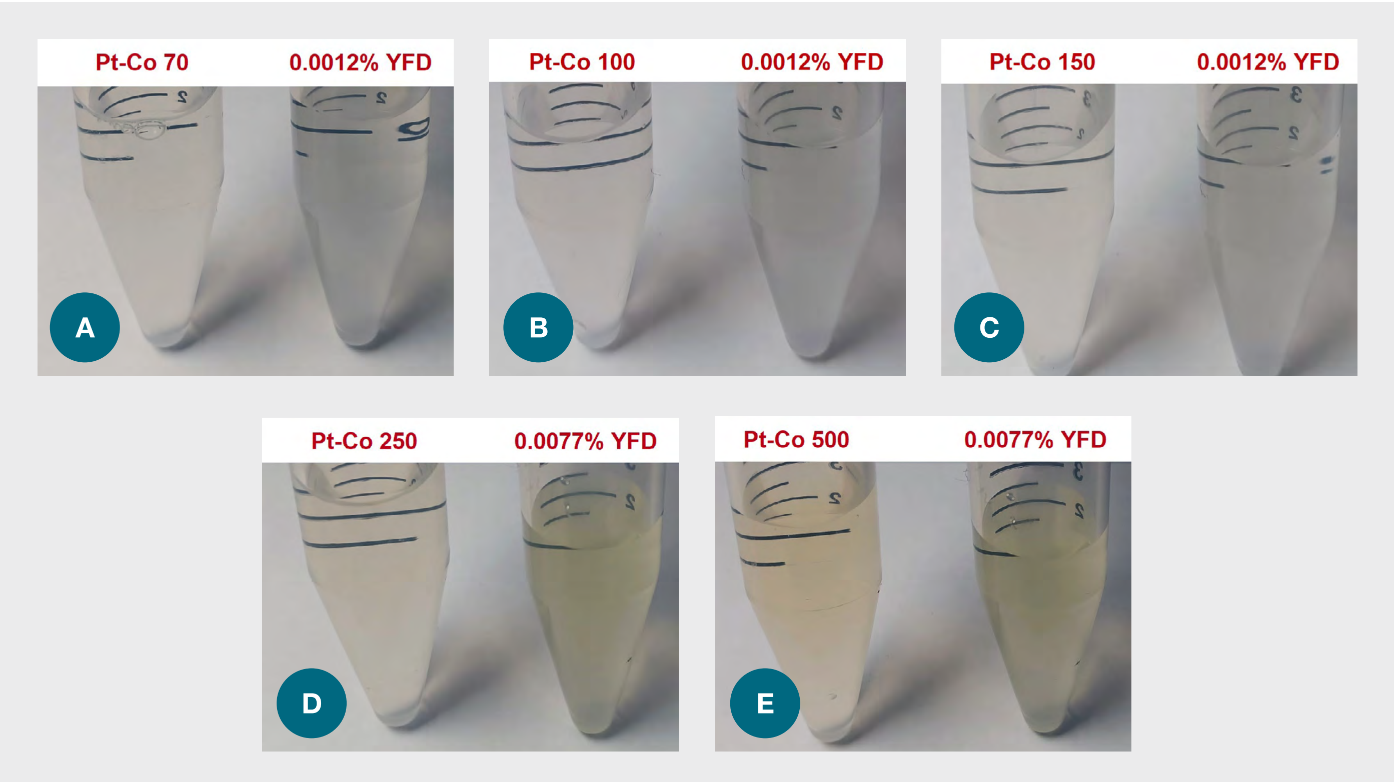

Figure 4: Images of (a-c) 0.0012% and (d,e) 0.0077% yellow food dye compared to various Pt-Co color standards.

Environmental samples

As indicated earlier, many water applications involve the use of the Pt-Co scale to assess color.[1,2,8] To demonstrate the ability to measure these samples, two separate examples of potential environmental specimens were prepared: a slurry consisting of dirt in water and a solution containing water and grass blades. The measured UV-Visible spectra are included in Figure 5a. For both sample sets, the absorption spectra are broad and virtually featureless. The spectrum of the dirt slurry exhibits an exponential increase in absorbance approaching shorter wavelengths while the spectrum of the grass samples appears to have a much shallower slope as the absorbance increases from long to short wavelength. Much like the yellow food dye samples, the spectra for both dirt and grass samples are very different from the Pt-Co color standards; nevertheless the samples still appear yellow to the eye.

Table 4 includes the calculated Pt-Co and yellowness index color values for both samples. As the dirt solution appears clearly yellow/brown through visual inspection (Figure 5b), the higher Pt-Co value matches expectation. However, it is difficult to tell how close the color of the filtered dirt solution is to the Pt-Co 350 standard as the latter does not have a brown tint to it. Consequently, using the Pt-Co color scale may not be the most appropriate method for establishing how yellow the solution appears, implying the yellowness index may be better in this circumstance.

For the grass solution, it is more difficult to tell the anticipated Pt-Co color value by eye, as shown in Figure 5c, as it appears much less vibrant than the filtered dirt solution. However, through analyzing the measured UV-Visible spectrum, as described previously, both a Pt-Co (90 ± 2) and yellowness index values (4.7 ± 0.1) could be determined. This result further establishes how color analysis through spectrophotometric techniques provides a more clear method for analyzing substances with minimal perceived color through visual inspection.

Sample	Pt-Co color value	Yellowness index (illuminant c, 2° observer)*
Filtered dirt solution	356 ± 2	17.8 ± 0.1
Grass sample in water	90 ± 2	4.7 ± 0.1

* Standard deviation calculated using the raw data.

Table 4: Pt-Co color values and yellowness index (illuminant C, 2° observer) determined for a filtered dirt sample and a grass sample.

Conclusions

The experiments included herein demonstrate the ability to determine both the Pt-Co and yellowness index color values for a variety of samples though UV-Visible absorption measurements. The results for the standardized Pt-Co solutions, obtained using the Evolution One spectrophotometer and accompanying Insight Pro software, detail the accuracy for measurements determined using a 1.0 cm path length. For other samples, like food dye or plant/soil samples, though the UV-Visible spectra differ from the Pt-Co standards, both Pt-Co and yellowness index color values could be readily calculated. Additionally, the results herein demonstrate how UV-Visible color analysis can aid in color determination in those circumstances where the yellowness is difficult to perceive by eye. In this way, UV-Visible measurements can help take the guesswork out of color analysis.

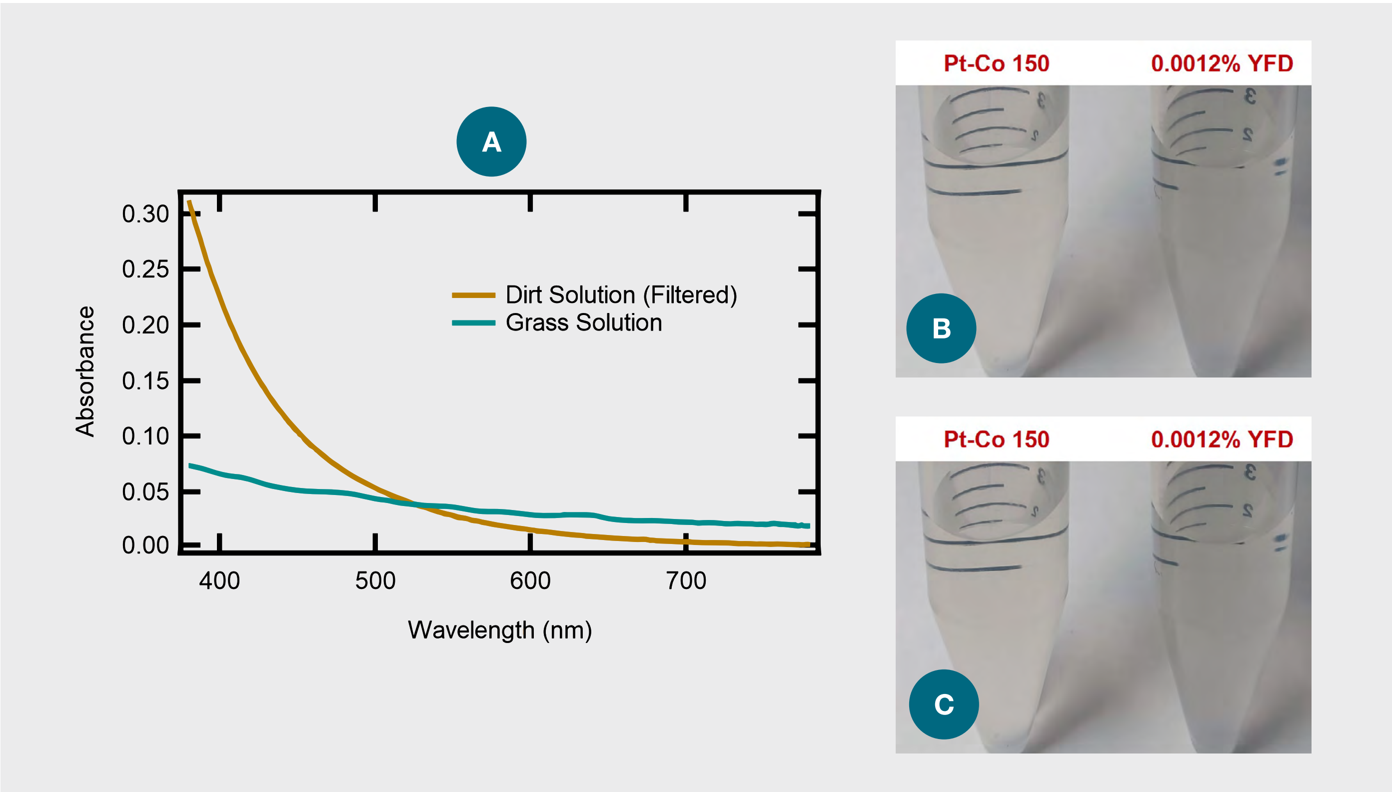

Figure 5: (a) UV-Visible spectra of a grass solution (blue) and a filtered dirt solution (orange). Images of (b) a filtered dirt solution and (c) a grass solution compared against a Pt-Co 350 and Pt-Co 100 color standard, respectively.

References

1. Coro, E.; Laha, S., Color Removal in Groundwater Through the Enhanced Softening Process, Water Res., **2001**, 35, 1851 – 1854.
2. Zobkov, M. B.; Zobkova, M. V., New Spectroscopic Method for True Color Determination in Natural Water with High Agreement with Visual Methods, Water Res., **2020**, 177, 115773.
3. ASTM International. Standard Test Method for Color of Clear Liquids (Platinum-Cobalt Scale); ASTM D1209-00; West Conshohocken, PA.
4. International Organization for Standardization. Clear Liquids – Estimation of Colour by the Platinum-Cobalt Colour Scale; ISO 6271:2015; Geneva, Switzerland.
5. ASTM International. Standard Practice for Computing the Color of Objects by Using the CIE System; ASTM E308-08; West Conshohocken, PA.
6. ASTM International. Standard Practice for Calculating Yellowness and Whiteness Indices from Instrumentally Measured Color Coordinates; ASTM E313-20; West Conshohocken, PA.
7. Color Analysis for Pharmaceutical Products Using UV-Visible Absorption Techniques, https://assets.thermofisher.com/TFS-Assets/MSD/Application-Notes/color-analysis-pharmaceutical-an56364-en.pdf, 2022.
8. Standard Methods for the Examination of Water and Wastewater, 23rd ed.; Baird, R. B.; Eaton, A. D.; Rice, E. W., Eds; American Public Health Association, **2017**.

Learn more at **thermofisher.com/uv-vis**