Ensuring Precision and Efficiency in Pharmaceutical Color Assessment: Variable Pathlength Technology Multiattribute Approach Under EP 2.2.2

White Paper

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Abstract

Current methods for color assessment in pharmaceutical solutions, particularly visual methods, suffer from significant subjectivity, with results varying based on observer perception, lighting conditions, and experience. Single-pathlength colorimeters, although more objective, acquire a single datapoint that requires a baseline correction. Given the inability to verify if the detector is operating within its linear range, these limitations lead to inaccurate or unreliable readings, complicating quality control.

This study compares the CTech™ SoloVPE® Variable Pathlength Technology (VPT) System and the HunterLab Vista® colorimeter, demonstrating VPT is capable of consistent, reliable color measurements compliant with EP Chapter 2.2.2 guidelines. VPT optimizes pathlength based on optical density, reducing sample volume requirements from 1–2 mL to just 120 µL. Its built-in linearity check and multiattribute testing enhance reliability and efficiency.

Key findings indicate that VPT outperforms traditional single-pathlength instruments by acquiring high-quality data from multiple points.

Introduction

The assessment of the degree of color in pharmaceutical solutions, notably monoclonal antibody (mAb) solutions, is a critical aspect of quality control, as mandated by the European Pharmacopoeia (EP) Chapter 2.2.2. Recognizing color as a critical quality attribute ensures the safety, efficacy, and consistency of pharmaceutical products throughout their shelf life. Variations in color can signal conditions like oxidation, degradation, or contamination, potentially affecting therapeutic effectiveness. Examples of the complex factors at play include UV light exposure causing tryptophan oxidation (resulting in yellow/yellow-brown discoloration) and the inclusion of vitamins like B12, B2, or C during cell culture leading to red/pink, yellow, or brown coloration, respectively. This underscores the paramount importance of meticulous color assessment during batch release and stability studies to identify deviations from quality standards, indicating potential stability issues or the presence of impurities. [1]

The EP Chapter 2.2.2 outlines both visual and instrumental methods for assessing the color of pharmaceutical substances. The visual method, while simple and quick, relies on human perception, making it subjective and inconsistent across different observers. Disadvantages include variability in lighting conditions, observer experience, and color perception, leading to less precise and reproducible results. In contrast, instrumental methods, using spectrophotometry, offer objective, quantifiable, and reproducible data, significantly reducing the subjectivity associated with visual assessments. [2]

The instrumental approach aims to replicate the human eye's perception of colors through the utilization of tristimulus values, which quantify the intensity of three primary colors: red, green, and blue. The international standard ISO/CIE 11664 outlines the procedure for assessing tristimulus values X, Y, Z from transmittance measurements within the 400–700 nm range. Tristimulus values are subsequently translated into color coordinates L*, a*, and b* through a conversion process. In the CIELAB color space, L* represents lightness ranging from 0 (black) to 100 (white), while a* and b* represent the chromaticity coordinates on the green-red and blue-yellow axes, respectively. These color coordinates provide a perceptually uniform

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representation of color, enabling accurate and consistent color comparison and assessment across different materials and lighting conditions. [3]

In this study, we demonstrate that EP-compliant color assessment extends beyond conventional single-pathlength colorimeters and can be performed with variable pathlength technology (VPT). The SoloVPE instrument optimizes the pathlength with respect to the sample's optical density and incorporates an automatic linearity check, providing real-time verification of its operation within the detector's linear range. This feature validates the accuracy of slope regression measurements and simplifies quality control with a straightforward pass/fail metric. Moreover, the SoloVPE System allows for a significant reduction in sample volume; measurements that typically require 1–2 mL can be performed with only 50–120 µL, minimizing sample consumption and enabling the analysis of precious or limited samples. Additionally, the SoloVPE System allows for GMP-grade concentration readings, facilitating multiattribute testing in a single measurement. The multiattribute approach streamlines workflows by reducing the time and resources required for individual tests, while ensuring the safety, efficacy, and consistency of products through the detection of multiple impurities or deviations from specifications, making it an essential tool in quality control.

> ***"The SoloVPE System allows for GMP-grade concentration readings, facilitating multiattribute testing in a single measurement."***

Methodology

Below, we outline the method prescribed by EP 2.2.2 and ISO/CIE 11664. This method involves obtaining transmittance values across the 400–700 nm spectrum, typically using a 10 mm pathlength. In contrast, a VPT instrument captures multiple absorbance readings across a range of pathlengths, providing an output in units of [Abs/mm], known as "slope." Generally, this slope value can be converted into the corresponding absorbance value for a single pathlength, by multiplying the slope by the pathlength in millimeters. We demonstrate the validity of this conversion and substantiate it by comparing color coordinates L*, a*, and b* and the determined coloration of EP-reference solutions and mAb samples using a HunterLab Vista colorimeter (1 cm cuvette) and SoloVPE System.

1) To acquire tristimulus values X, Y, and Z of a sample, EP 2.2.2 provides standardized values for a 2° angle and diffuse daylight (illuminant C):

   - Color-matching functions x_λ, y_λ, and z_λ given by CIE 11664/1:2019 for standard observer CIE 1931 (2° angle)
   - Relative spectral power distribution S_λ of the CIE illuminant C
   - A normalizing constant k is calculated as follows:

$$k = \frac{100}{\sum_{\lambda} S_\lambda \bar{y}_\lambda \Delta\lambda}$$

   Where $\Delta\lambda$ is the wavelength step size (e.g. 5 nm) with which the transmittance values were acquired.

2) Tristimulus values X, Y, and Z are calculated with the following equations:

$$X = k \sum_{\lambda} T_\lambda \bar{x}_\lambda S_\lambda \Delta\lambda$$

$$Y = k \sum_{\lambda} T_\lambda \bar{y}_\lambda S_\lambda \Delta\lambda$$

$$Z = k \sum_{\lambda} T_\lambda \bar{z}_\lambda S_\lambda \Delta\lambda$$

   Where T_λ is the acquired transmittance value at wavelength λ in the 400–700 nm range.

   Slope values were collected using five pathlengths: 0.2, 1.5, 2.7, 3.9, and 5.0 mm, which were selected in order to keep the absorbance within the detector's linear range for compliance with the Beer-Lambert law:

a) A maximum pathlength of 5 mm was used to avoid detector saturation for dark (Y1 and B1) reference samples at 400 nm (0.4–0.5 Abs/mm slope)

b) A minimum pathlength of 0.2 mm was chosen to maximize the slope regression coefficient (R^2) for the lightest (Y7 and B9) reference samples.

120 µL of sample is prepared in a plastic vessel for each measurement. Data is acquired in 5 nm wavelength steps between 400 and 700 nm, with a 1-second averaging time. Acquired slope values, *m*, are subsequently converted to transmittance using $T_\lambda = 10^{(2\text{-}m\times10)}$ for each wavelength *λ*.

3) To convert tristimulus values into color coordinates L*, a*, and b*, the following equations are used:

$$L^* = 116 f(Y/Y_n) - 16$$

$$a^* = 500\left[f(X/X_n) - f(Y/Y_n)\right]$$

$$b^* = 200\left[f(Y/Y_n) - f(Z/Z_n)\right]$$

Where Y_n, X_n, Z_n are tristimulus values acquired for a de-ionized (DI) water sample.

Depending on the ratio of the tristimulus value of the sample (EP-reference solution or mAb solution) to the tristimulus value using water, f(X/Xn), f(Y/Yn), and f(Z/Zn) are given by:

$$f(X/X_n) = (X/X_n)^{1/3} \quad \text{if } (X/X_n) > (6/29)^3$$

$$f(X/X_n) = (841/108)(X/X_n) + 4/29 \quad \text{if } (X/X_n) \le (6/29)^3$$

and

$$f(Y/Y_n) = (Y/Y_n)^{1/3} \quad \text{if } (Y/Y_n) > (6/29)^3$$

$$f(Y/Y_n) = (841/108)(Y/Y_n) + 4/29 \quad \text{if } (Y/Y_n) \le (6/29)^3$$

and

$$f(Z/Z_n) = (Z/Z_n)^{1/3} \quad \text{if } (Z/Z_n) > (6/29)^3$$

$$f(Z/Z_n) = (841/108)(Z/Z_n) + 4/29 \quad \text{if } (Z/Z_n) \le (6/29)^3$$

4) The above equations describe how to assess the color coordinates L*, a*, and b* for the respective EP-reference solution and mAb-solutions. To assess the degree of color, the color difference between an EP-reference solution and test sample is calculated as follows:

$$\Delta E^*_{tr} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where ΔL* is the difference of color coordinates L* between an EP-reference solution and the sample.

After calculating ΔE^*_{tr} to each EP-reference solution within a chosen color series (e.g., yellow), the smallest ΔE^*_{tr} indicates the closest reference to the sample color. In Table 1 below, the smallest ΔE^*_{tr} (highlighted green) corresponds to EP-reference solution Y3. Thus, the test sample is closest to reference solution Y3.

Table 1. Example illustrating differential color coordinates between a sample and yellow references. The green highlight indicates the greatest similarity to the tested sample.

Test Sample vs. Reference	ΔL*	Δa*	Δb*	ΔE^*_{tr}
Y1	-0.13	6.31	-20.06	21.03
Y2	-1.22	5.14	-11.72	12.86
Y3	-1.94	3.86	-4.38	6.15
Y4	-3.11	-3.56	9.10	10.26
Y5	-3.32	3.56	-9.10	10.32
Y6	-3.65	-0.03	8.92	9.64
Y7	-3.67	-1.68	12.44	13.08

Results

Color coordinates L*, a*, and b* were acquired using the SoloVPE System for brown and yellow EP-reference solutions. Color difference ΔE^*_{tr} compared to DI water was calculated and plotted to verify the expected correlation with a 2nd degree polynomial fit (Figure 1, Figure 2).

Each graph, or calibration curve for the respective color series, confirms that higher concentration EP reference standards have greater color differences compared to water. For both tested color series, an $R^2 > 0.99$ was obtained.

Color coordinates and color difference were also acquired for the yellow color series using a USP<1061>-recommended

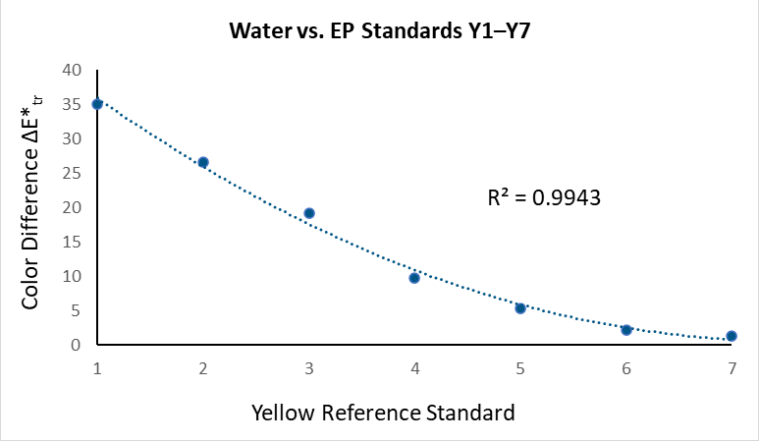

Figure 1. Color difference between water and yellow reference standard series using the SoloVPE System.

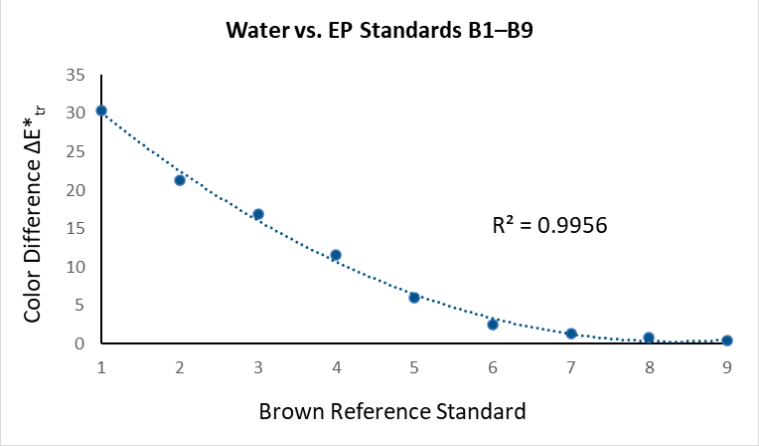

Figure 2. Color difference between water and brown reference standard series using the SoloVPE System.

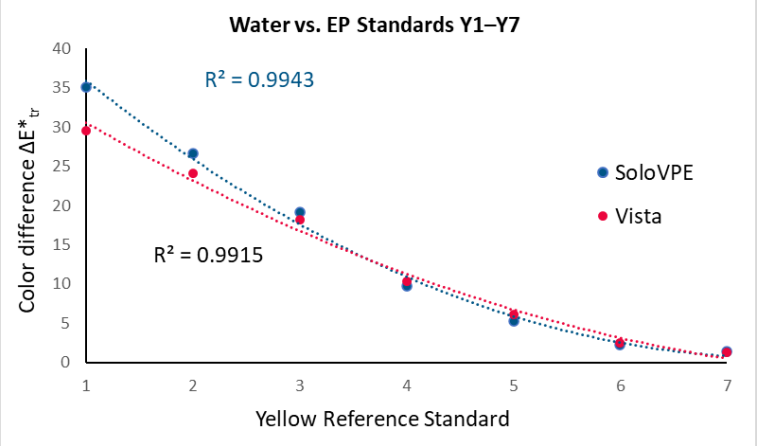

Figure 3. Color difference between water and yellow reference standard series. Measured with SoloVPE System and Vista.

colorimeter from HunterLab, the Vista spectrophotometer, and compared to the results obtained using the SoloVPE System. Both instruments yielded comparable data and correlation coefficients (R^2 > 0.99), but ΔE^*_{tr} diverged for the Y1 and Y2 reference standards (Figure 3).

For a more comprehensive analysis of systematic deviations in analyzing highly colorized samples, the output of the HunterLab Vista colorimeter was set to report values with a single decimal place. While this output is not EP compliant and is typically used in industries outside of biopharma, considering these values adds an important dimension to the discussion of Figure 3.

Despite an expected output of Y1.0 and Y2.0 for a Y1 and Y2 reference standard, respectively, column 2 in Table 2 illustrates that the degree of coloration of these standards is underestimated (Y1.6 and Y2.3) by the HunterLab Vista instrument. Since the color assessment is based on a relative comparison between the tested and reference samples, this does not indicate the results are incorrect. However, the reduced color difference between Y3 and Y2, as well as Y2 and Y1, lowers the resolution of the color assessment.

In contrast, the SoloVPE variable pathlength method, which optimizes pathlengths with respect to optical density, remains accurate when single-pathlength instruments become imprecise. Common factors contributing to inaccurate single-pathlength measurements include decreased light source intensity, decreased detector sensitivity, or the sample's optical density falling outside of the detector's linear range.

However, the color coordinates for Y3–Y7 obtained from both instruments are in good alignment, confirming that the SoloVPE System's slope values can be converted to transmittance values and subsequently to color coordinates. As color coordinates are absolute values, they can be utilized for comparability studies between instruments and batch-to-batch testing.

The color coordinates obtained from the SoloVPE System were subsequently converted into color difference ΔE^*_{tr} and the degree of coloration, following the methodology outlined in the Methodology section and EP 2.2.2. These results are then compared with the EP-compliant output from HunterLab Vista (Table 3).

Table 2. Degree of Color measured with Vista and color coordinates L, a*, b* measured with the SoloVPE System for the respective yellow reference standard. Red highlighted cells indicate where the degree of coloration was underestimated.*

EP reference standard	Vista Color Degree	SoloVPE L*	Vista L*	SoloVPE a*	Vista a*	SoloVPE b*	Vista b*
Y1	Y1.6	96.27	96.89	-8.49	-5.47	33.80	28.72
Y2	Y2.3	97.36	97.77	-7.32	-5.39	25.46	23.29
Y3	Y3.2	98.07	98.74	-6.04	-4.79	18.12	17.41
Y4	Y4.1	99.25	99.63	-3.55	-3.21	9.10	9.78
Y5	Y5.0	99.46	99.98	-2.15	-2.03	4.82	5.64
Y6	Y5.9	99.78	100.32	-0.82	-0.92	2.05	2.29
Y7	Y6.9	99.80	100.46	-0.50	-0.47	1.30	1.09

As observed from Table 3, the EP-compliant color assessments of yellow reference standards are consistent across both instruments for Y1–Y5. However, discrepancies arise in the output for the Y6 and Y7 standards from the HunterLab Vista colorimeter, indicating a vulnerability in accuracy when employing decimal places. Even a minor variability of ±0.1 could potentially stem from instrumental inaccuracies, consequently impacting the resulting EP-output. Furthermore, companies must make a deliberate decision regarding whether to truncate or round decimal places. To mitigate such uncertainties, EP chapter 2.2.2. clearly outlines the utilization of color difference ΔE^*_{tr}, and Table 3 affirms that this approach yields an accurate assessment of the degree of color within the yellow standard series.

Table 3. Degree of coloration obtained with SoloVPE and Vista Systems for yellow EP reference standards.

Reference standard	SoloVPE System	Vista (EP output)	Vista (raw output)
Y1	Y1	Y1	Y1.6
Y2	Y2	Y2	Y2.3
Y3	Y3	Y3	Y3.2
Y4	Y4	Y4	Y4.1
Y5	Y5	Y5	Y5.0
Y6	Y6	Y5	Y5.9
Y7	Y7	Y6	Y6.9

To illustrate real life applications in pharmaceutical industry, nine distinct drug products containing monoclonal antibodies (mAbs), constituting a dilution series, were assessed for their degree of color within the yellow and brown color spectra (Table 4). A permissible variation of ±0.2 is adopted, with acceptable outcomes highlighted green and deviations in red. Both the EP-compliant output as well as the output with one decimal place are displayed. For comparison, results from a visual assessment adhering to EP 2.2.2 requirements for three samples are shown in column 6.

As depicted in Table 4, the output of both instruments closely aligns for the majority of samples, further validating the efficacy of the developed method not only for standards but also for real biopharmaceutical samples. Particularly noteworthy is the Y0.1 output observed in highly colorized samples, indicating that the optical density was too high to accurately assess the degree of color with a 1 cm pathlength. In contrast, the SoloVPE System features a built-in algorithm that adjusts the pathlength based on the optical density, resulting in accurate readings. Additionally, visual assessment results were available for three samples. While the degree of color was assessed identically for one sample using the visual and SoloVPE methods, discrepancies between the visual assessment and the objective instrumental measurements highlight the subjectivity inherent in visual assessment methods.

Table 4. Degree of coloration obtained with SoloVPE and Vista Systems for 8 mAb samples and dilutions of mAb 8. Degree of coloration is assessed in reference to yellow and brown series standards. SoloVPE results are colored green to illustrate agreement with the Vista EP Output or with the Vista Raw Output ±0.2.

Sample	Vista EP Output (Raw Output)	SoloVPE System Result	Vista EP Output (Raw Output)	SoloVPE System Result	Visual Assessment
mAb1	Y3 (Y3.8)	Y3	B3 (B3.9)	B3	B2
mAb2	Y3 (Y3.9)	Y4	B4 (B4.0)	B3	B3
mAb3	Y3 (Y3.4)	Y3	B3 (B3.2)	B3	B2
mAb4	Y5 (Y5.8)	Y6	B5 (B5.8)	B6	
mAb5	Y1 (Y1.8)	Y2	B1 (B1.6)	B1	
mAb6	Y1 (Y1.7)	Y2	B1 (B1.6)	B1	
mAb7	Y0.1	Y1	B0.1	B1	
mAb8-dilution	Y0.1	Y1	B0.1	B1	
mAb8-dilution	Y0.1	Y1	B0.1	B1	
mAb8-dilution	Y1 (Y1.9)	Y2	B1 (B1.8)	B1	
mAb8-dilution	Y3 (Y3.2)	Y3	B2 (B2.9)	B2	
mAb8-dilution	Y3 (Y3.9)	Y3	B3 (B3.8)	B3	
mAb8-dilution	Y6 (Y6.4)	Y6	B6 (B6.7)	B7	
mAb8-dilution	Y7 (Y7.5)	Y7	B9 (B9.0)	B8	
mAb8-dilution	Y7 (water)	Y7	B9 (water)	B9	

> ***"The SoloVPE System is user friendly, reducing complexity and optimizing training time for operators since multiple attributes can be obtained from the same sample preparation, device, and interface."***

Summary

The comparative assessment of color degree using the SoloVPE System and HunterLab Vista yielded consistent results, reinforcing the SoloVPE System's precision and reliability in color measurement. Most notably, the developed method aligns strictly with EP Chapter 2.2.2 guidelines, showcasing SoloVPE System's capability to adhere to regulatory standards. The standout feature of the SoloVPE System is its capability for multiattribute testing in a single operation, including both color and concentration. This significantly simplifies the analytical process, improves efficiency, and accelerates the comprehensive quality control timeline. This functionality, combined with the SoloVPE System's capacity to generate high-quality data by leveraging multiple data points per measurement, emphasizes its superior performance in accurate and reliable color analysis. The system also eliminates the need for background correction and introduces advanced mechanisms for scatter correction, simplifying future method development for analyzing turbid samples. Moreover, SoloVPE System's design significantly lowers the required sample volume for analysis, from 1–2 mL down to just 120 µL, conserving valuable samples and minimizing waste—a crucial advantage for personalized medicines like cell and gene therapies.

The inclusion of a built-in linearity check ensures that the system's operation is constantly verified within the detector's linear range, adding an extra layer of reliability to slope-based measurements. Additionally, the SoloVPE System is user-friendly, reducing complexity and optimizing training time for operators since multiple attributes can be obtained from the same sample preparation, device, and interface. Furthermore, the technology is already compatible with GMP guidelines for protein content measurements and can be reliably

configured for compliance. The SoloVPE System also offers GMP-level accuracy with its ability to validate and lock down methods, enhancing the robustness of quality control processes. Moreover, the SoloVPE System's applications extend beyond this specific method, making it a versatile device suitable for multiple modalities.

Altogether, the integration of these advanced features makes the SoloVPE System a more streamlined, accurate, and versatile tool for color assessment, vital for maintaining product quality and meeting regulatory compliance within the pharmaceutical industry. This also translates into significant cost efficiency for pharmaceutical companies, as the multiattribute capability reduces the need for additional instruments and maintenance and minimizes the amount of wasted samples. The objective nature of the SoloVPE System's measurements further enhances the quality of the method, eliminating subjectivity and ensuring consistent, reproducible results.

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