Technical Overview

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Absorbance Accuracy and Linear Dynamic Range to 3 Abs and beyond with the Agilent Cary 3500 UV-Vis Spectrophotometer

The photometric accuracy and linear dynamic range of the Agilent Cary 3500 can be demonstrated to be up to 3.5 Abs using different concentrations of potassium dichromate ($K_2Cr_2O_7$) according to the guidelines described in the United States Pharmacopeia (USP).

The photometric accuracy and linearity of a spectrophotometer determine its ability to measure the absorbance of compounds, which is directly related to known absorptivity or concentration.

Photometric accuracy determines how accurately the instrument measures the absorbance of a sample. Poor photometric accuracy will produce incorrect results.

Photometric linearity determines how accurately an instrument measures absorbance with increasing concentration. Poor photometric linearity will also produce incorrect results.

All tests for these two parameters rely on the Beer-Lambert law, which dictates that a linear relationship exists between absorbance and sample concentration (refer to Figure 1). The simple linear relationship between absorbance and concentration and the relative ease of measurement of UV-visible light have made UV-visible spectroscopy the basis for thousands of quantitative analytical methods.

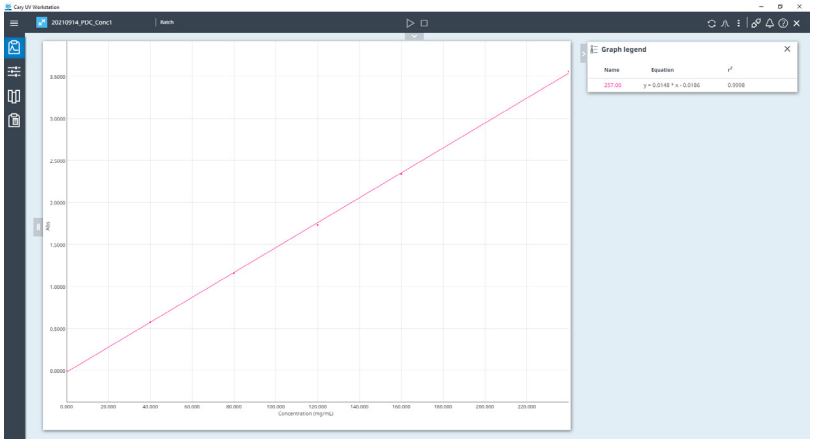

Figure 1. A screen capture from the Cary UV Workstation software, showing the potassium dichromate calibration curve at 257 nm from 40 to 240 mg/L. At the highest concentration, the absorbance is above 3.5 Abs. The calibration curve has a R2 of 0.9998.

The dynamic range over which the spectrophotometer remains linear is also important. A linear dynamic range allows the analysis of highly turbid samples, various sample concentrations, and decreases time required for sample preparations (dilutions).

The photometric accuracy and linear dynamic range of the Agilent Cary 3500 can be demonstrated using different concentrations of potassium dichromate ($K_2Cr_2O_7$) according to the guidelines described in the United States Pharmacopeia (USP).

Photometric accuracy

Photometric accuracy tests were conducted using potassium dichromate ($K_2Cr_2O_7$) certified reference materials. The USP method to test photometric accuracy in the UV region of the spectrum requires a solution of potassium dichromate ($K_2Cr_2O_7$) in dilute perchloric acid to be measured. The absorbance at 235 nm, 257 nm, 313 nm, and 350 nm is then determined. When below 1 Abs, the absorbance accuracy must be ± 0.01 Abs, and when above 1 Abs, the accuracy must be ± 1 % of the absorbance measured.

Table 1 shows the results of measuring five different potassium dichromate certified reference materials. All the results were within the accuracy required by the USP.

Table 1. Photometric accuracy using certified potassium dichromate $K_2Cr_2O_7$ from 40 to 240 mg/L. A solution of 0.001M Perchloric acid was used as a blank and the reference beam was left empty during measurements.

Potassium Dichromate ($K_2Cr_2O_7$) 40 mg/L					
Wavelength (nm)	Certified value	Cary 3500 value (n=6)	Difference between measured and certified values	Accuracy Tolerance	Typical Results
235.00	0.4931	0.4973	0.0042	±0.010 Abs	Pass
257.00	0.5704	0.5779	0.0075	±0.010 Abs	Pass
313.00	0.1914	0.1973	0.0059	±0.010 Abs	Pass
350.00	0.4239	0.4336	0.0097	±0.010 Abs	Pass
Potassium Dichromate ($K_2Cr_2O_7$) 80 mg/L					
Wavelength (nm)	Certified value	Cary 3500 value (n=6)	Difference between measured and certified values	Accuracy Tolerance	Typical Results
235.00	0.9995	1.0029	0.0034	±0.010 Abs	Pass
257.00	1.1562	1.1627	0.0065	±1 %	Pass
313.00	0.3874	0.3961	0.0087	±0.010 Abs	Pass
350.00	0.8559	0.8659	0.0100	±0.010 Abs	Pass

Potassium Dichromate ($K_2Cr_2O_7$) 120 mg/L					
Wavelength (nm)	Certified value	Cary 3500 value (n=6)	Difference between measured and certified values	Accuracy Tolerance	Typical Results
235.00	1.4974	1.4915	-0.0059	±1 %	Pass
257.00	1.7352	1.7319	-0.0033	±1 %	Pass
313.00	0.5751	0.5827	0.0076	±0.010 Abs	Pass
350.00	1.2753	1.2807	0.0054	±1 %	Pass
Potassium Dichromate ($K_2Cr_2O_7$) 160 mg/L					
Wavelength (nm)	Certified value	Cary 3500 value (n=6)	Difference between measured and certified values	Accuracy Tolerance	Typical Results
235.00	2.0188	2.0141	-0.0047	±1 %	Pass
257.00	2.3451	2.3439	-0.0012	±1 %	Pass
313.00	0.7716	0.7791	0.0075	±0.010 Abs	Pass
350.00	1.7147	1.7209	0.0062	±1 %	Pass
Potassium Dichromate ($K_2Cr_2O_7$) 240 mg/L					
Wavelength (nm)	Certified value	Cary 3500 value (n=6)	Difference between measured and certified values	Accuracy Tolerance	Typical Results
235.00	3.0867	3.0747	-0.0120	±1 %	Pass
257.00	3.5899	3.5886	-0.0013	±1 %	Pass
313.00	1.1686	1.1768	0.0082	±1 %	Pass
350.00	2.5988	2.6091	0.0103	±1 %	Pass

Photometric linearity

To verify photometric response (linearity), the United States Pharmacopeia (USP43-NF 38), Chapter <857> Ultraviolet-visible spectroscopy states:

"Verification of photometric response (linearity) is required, and it should be evaluated using a standard type appropriate for the wavelength(s) required, where at least three different absorbance levels appropriate to and spanning the required operational range are measured. Given the above requirement, it is not necessary to calculate the correlation coefficient of the standard response to demonstrate linearity; just demonstrate that at the different absorbance levels selected the acceptance limits have been met." which is demonstrated in (Table 1). (*1*)

The calibration curve generated from the five potassium dichromate standards using the Cary 3500 had an R^2 value of 0.9998 for 235 and 257 nm and 0.9999 for 313 and 350 nm. As shown in Figure 2, the Cary 3500 has excellent photometric linearity beyond 3 Abs. Good photometric linearity means that highly concentrated liquid samples can be measured accurately, without dilution.

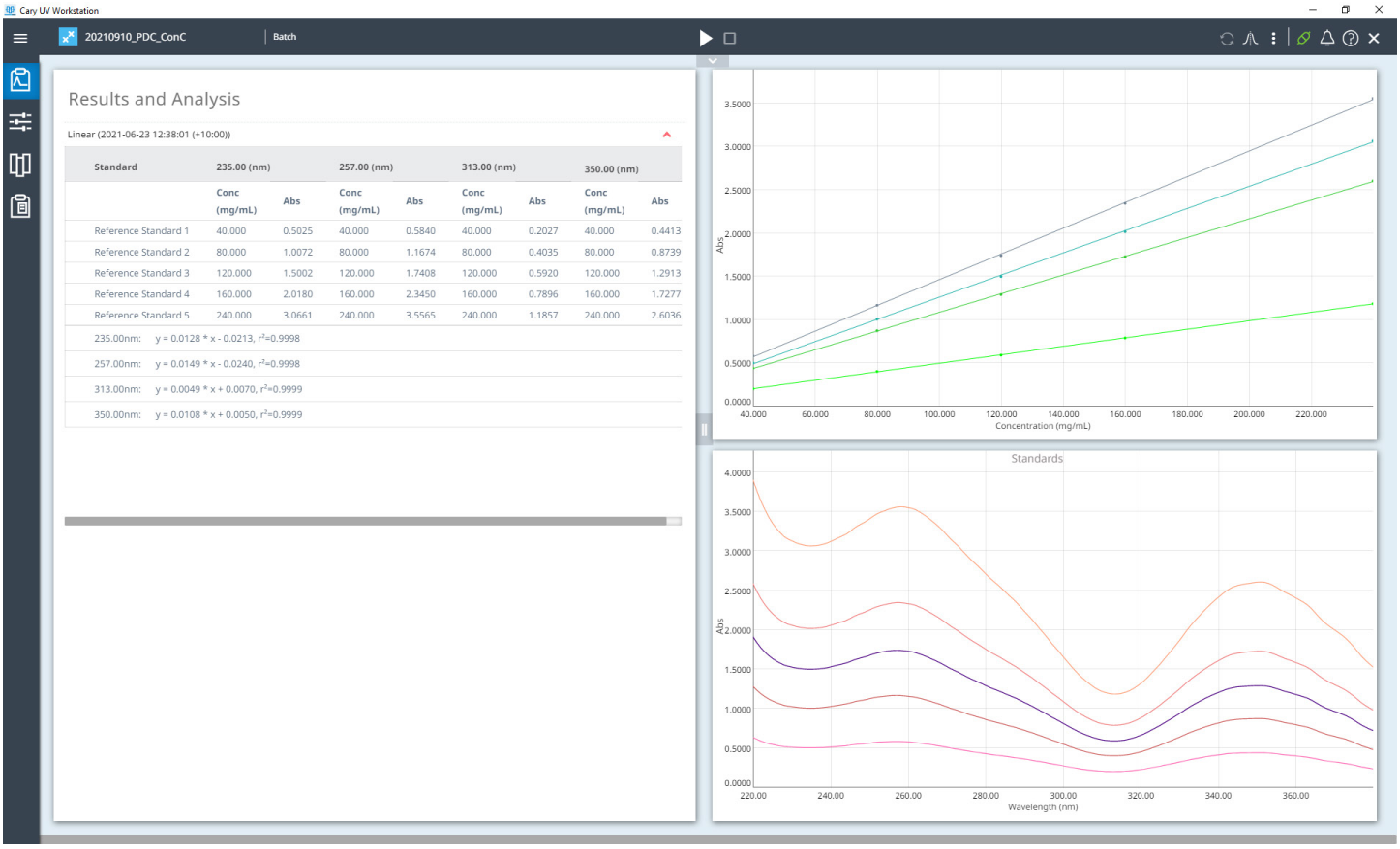

Figure 2. A screen capture from the Cary UV Workstation software, showing the potassium dichromate calibration curves (top right) at 235, 257, 313 and 350 nm along with the associated wavelength scans for the standards (bottom right). On the left, the linear equations and the correlation coefficients can be seen along with the raw values at 235, 257, 313 and 350 nm.

The plot of absorbance vs concentration (mg/L) shows the wide dynamic range and confirms that quantitative analysis of potassium dichromate from 40 to 240 mg/L is at the peak absorption wavelength of 257 nm. At the highest potassium dichromate concentration, the absorbance at this wavelength exceeds 3 Abs.

Reference

Chapter <857> Ultraviolet-Visible Spectroscopy . United States Pharmacopeia and National Formulary (USP43-NF38 - 7166). DocID: GUID-4C5C1937-524A-4BED-95E7-384EDE3745E0_3_en-US

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