Technical Overview

Comparing the Performance of the Cary 3500 and Cary 8454 UV-Vis Spectrophotometers

For sample quantification measurements

Introduction

Agilent launched the instrument of choice for UV-Vis measurements for the global pharma industry in the 1980s with the introduction of a diode array spectrophotometer. This technology was revolutionary and brought simplicity, speed, and robustness to quality assurance laboratories. Around the same time, Cary UV-Vis spectrophotometers continued to ascend as a benchmark of performance in academic and research institutes, underpinned by their double out-of-plane Littrow monochromator optical design.

In 2018, Agilent launched the Cary 3500 by combining the foundational research grade optics of the Cary brand with a revolutionary enhancement to user workflow. The benefits of a robust, nonmoving, multicell for simultaneous measurements have been realized for increased throughput and preservation of the integrity of a

UV analysis. The Cary 3500 uses a long-life xenon flash lamp as the light source and a research-grade double out of plane Littrow monochromator to offer photometric performance advantages over the Cary 8454. The software controlling the instrument has also been newly developed to simplify workflows and create a better user experience.

Good manufacturing practice (GMP) guidelines require method transfer equivalence to be demonstrated when migrating a method from one instrument to another. This practice ensures that results are reproducible and comparable. This technical note discusses the transfer of a well-established method, the quantification of a potassium dichromate solution, from the Cary 8454 to a Cary 3500. Advantages offered by the Cary 3500 for such a measurement are also discussed.

Experimental

Equipment

- Cary 8454 UV-visible spectrophotometer, controlled by UV-Vis ChemStation software
- Cary 3500 UV-visible spectrophotometer, controlled by Cary UV Workstation software

Reagents

Certified standard solutions of potassium dichromate were used to create a calibration curve. Another certified standard solution of potassium dichromate was used as a sample. The details are shown in the table following:

Solution	Contents
Blank Solution	0.001 M perchloric acid
Certified Standard Solutions used for Calibration	40, 80, 120, 160, 200 and 240 mg/L potassium dichromate in 0.001 M perchloric acid
Certified Solution, used as a Sample	60 mg/L potassium dichromate in 0.001 M perchloric acid

Part 1. Determining the analysis wavelength

Although the wavelength associated with the absorbance peak of potassium dichromate is well documented and stable, performing a wavelength scan to confirm this is good practice. In other situations, the peak wavelength may shift due to interactions between chemical components, changes in pH or other environmental changes. A quick survey scan can highlight any changes to the expected state of the sample. The Cary 8454 collected a spectrum with each measurement so this information was always available for every sample. The Cary 3500 scan rate is fast enough that performing sample scans routinely is practical. Using the 60 mg/L potassium dichromate sample, a wavelength scan was performed using the parameters indicated in Table 1.

Table 1. Instrument parameters used for a wavelength scan and peak analysis.

Parameter	Setting	
	Cary 8454	Cary 3500
Measurement Mode	Standard mode, Spectrum/ Peaks Task in UV-Vis ChemStation software	Scan mode, in Cary UV Workstation software
Analytical Tool	Peal/Valley Find – Up to Two Peaks	Peak/Valley Find – Threshold 0.1
Wavelength Range	190 to 600 nm (display)	190 to 1100 nm
Data Interval	1.0 nm	1.0 nm

Method

The Cary 8454 was switched on and lamps allowed to warm up for one hour. The Cary 3500 uses a xenon flash lamp and so does not require warm up. A baseline was established on both systems, using the perchloric acid blank solution. The 60 mg/L sample solution was then measured across the wavelength ranges indicated in Table 1.

Results

The same peaks were identified, with both the Cary 8454 and the Cary 3500 (Figure 1a and 1b), reporting the same wavelengths for the peaks.

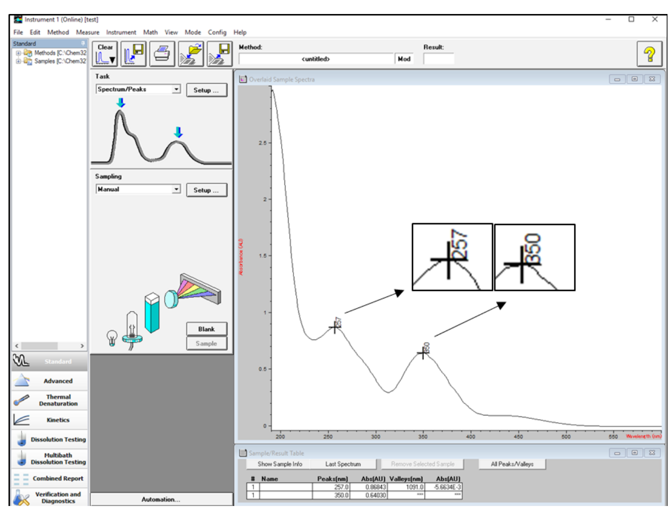

Figure 1a. Two peaks were reported by the Cary 8454 for the 60 mg/L potassium dichromate sample; 257 nm and 350 nm.

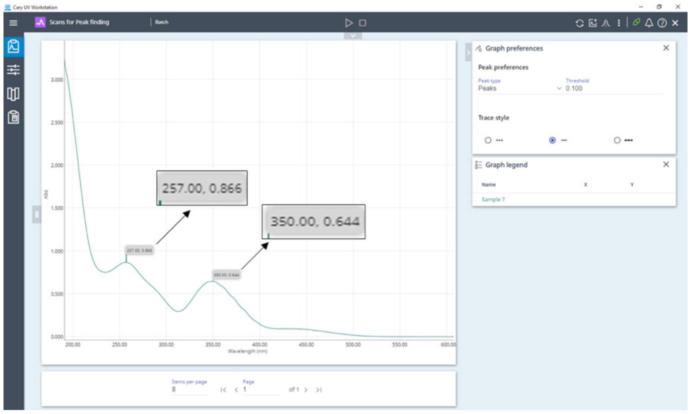

Figure 1b. Two peaks were reported by the Cary 3500 for the 60 mg/L potassium dichromate sample; 257 nm and 350 nm.

Part 2. Quantification of a sample

A calibration curve was prepared on both the Cary 8454 and the Cary 3500 instruments, using six potassium dichromate standards. The analysis wavelength selected for use was 257 nm. The parameters used are shown in Table 2 and Figure 2.

Table 2. Instrument parameters used for quantification.

Parameter	Setting	
	Cary 8454	Cary 3500
Measurement Mode	Standard mode, Quantification Task in UV-Vis ChemStation software	Concentration mode in Cary UV Workstation software
Wavelength	257 nm	
Calibration Curve Type	Linear	
Standards	6	
Sample	1	
Integration/Averaging Time	3.0 s	

Method

For both instruments, a baseline was performed using the perchloric acid blank. Both instruments measured the full spectrum for each of the standards and samples. On the Cary 8454 the standards and samples were measured one after the other. The Cary 3500 was able to measure all seven cuvettes simultaneously due to the design of the multicell module.

From the measured spectra, the absorbance at 257 nm for each of the standards was used to generate the calibration curve. The other certified solution was then measured as a sample.

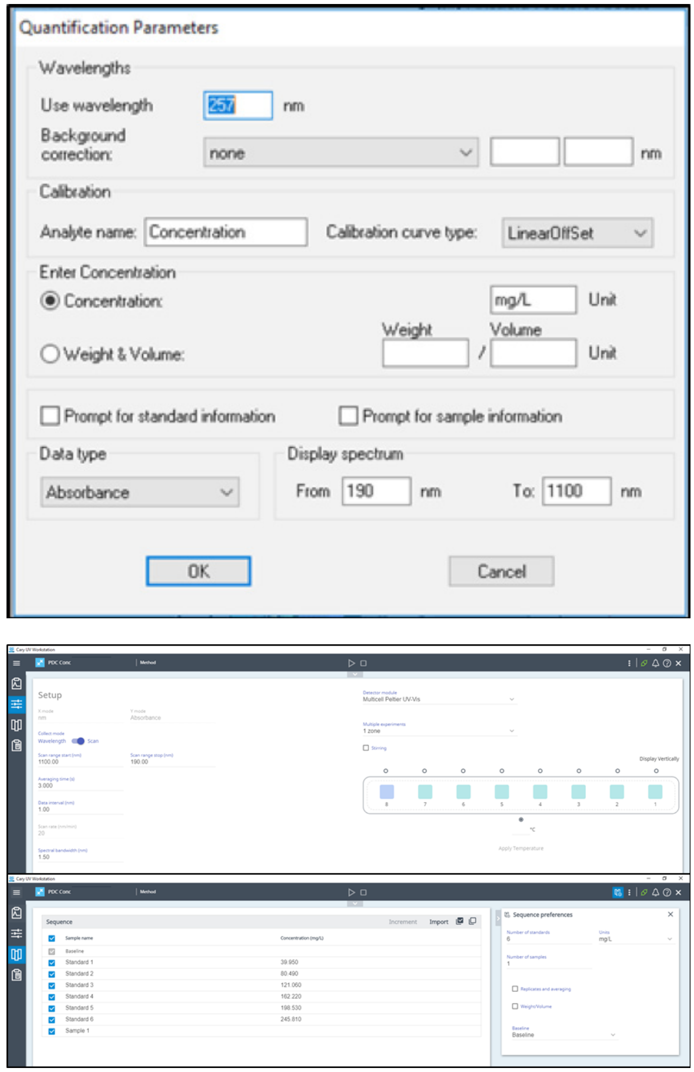

Figure 2. The measurement parameter setting screens for each instrument: Cary 8454 (upper), Cary 3500 (lower).

Results

A calibration curve was generated on each instrument. The calibration curve prepared via the Cary 8454 had a correlation coefficient (R^2) of 0.9999 (Figure 3). The concentration of the potassium dichromate sample was determined to be 61.37 mg/L (Table 3). This result compares to the certified concentration of 60.73 mg/L. The calibration curve prepared via the Cary 3500 also had a correlation coefficient (R^2) of 0.9999. The concentration of the potassium dichromate sample was determined to be 60.72 mg/L (Table 3).

The measured absorbance for each of the certified standard solutions is shown in Table 3. The two instruments demonstrated equivalent capability and also produced similar results. The absorbance of the two most concentrated standard solutions was approaching the upper measurement limit of the Cary 8454 around 3 Abs. The Cary 3500 has a photometric range of 4 Abs, so was able to measure these standard solutions more accurately.

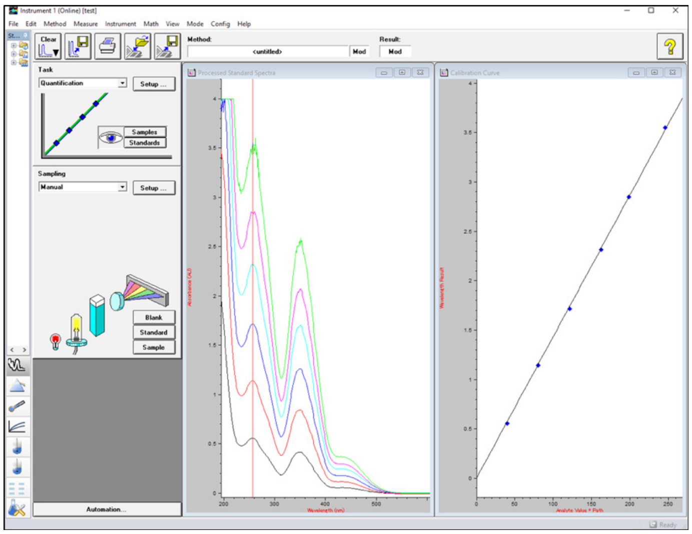

Figure 3. The wavelength scans of each standard solution and the calibration curve produced from the scans, using the Cary 8454.

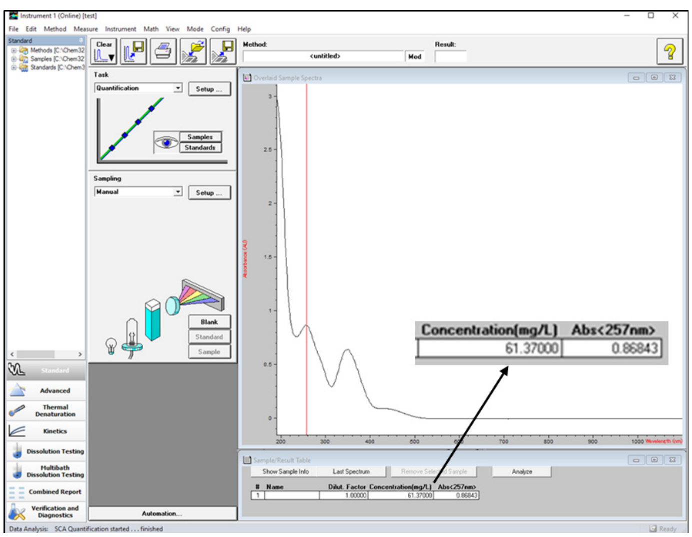

Figure 4. The sample result generated by the Cary 8454.

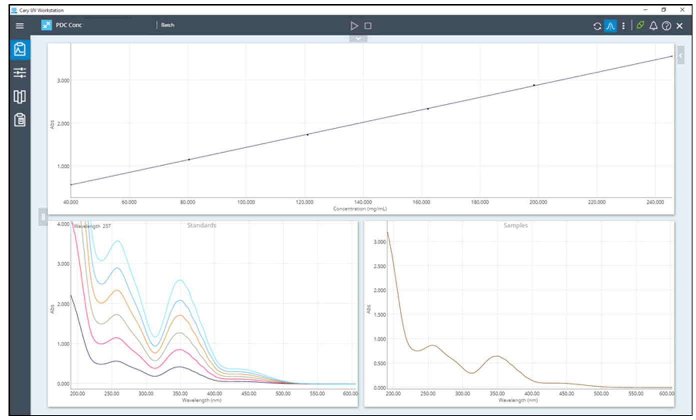

Figure 5. The absorbance spectra of the standards measured on the Cary 3500, and the calibration curve produced from the absorbance at 257 nm. The sample absorbance scan is shown in the bottom-right window.

Table 3. Absorbance data for the certified standards and the sample solution, as measured on both the Cary 8454 and the Cary 3500.

Sample	Certified Values (Abs)*	Cary 8454 Measured Values	Error (Abs)	Cary 3500 Measured Values	Error (Abs)
Std 1	0.5704	0.5627	0.0077	0.5703	0.0001
Std 2	1.1562	1.1456	0.0106	1.1566	−0.0004
Std 3	1.7352	1.7201	0.0151	1.7359	−0.0007
Std 4	2.3451	2.3276	0.0175	2.3442	0.0009
Std 5	2.9069	2.8697	0.0372**	2.8968	0.0101
Std 6	3.5899	3.5322	0.0577**	3.5583	0.0316
Sample	0.8673	0.8684	−0.0011	0.8686	−0.0013

** Certified absorbance at 257 nm*
***Error larger than 0.01 Abs or 1% when greater than 1 Abs*

Part 3. Timing comparison

The Cary 8454 is a diode array system and as such collects the entire 190 to 1100 nm spectrum in one acquisition. The Cary 3500 is a monochromator-based system where individual wavelengths are measured sequentially. Unlike other monochromator-based systems, the Cary 3500 has been designed to have a very fast wavelength change rate, allowing for data collection rates as fast as 150,000 nm/min.

In this comparison, the elapsed time required to acquire a scan of a sample across the wavelength range of 190 to 1100 nm, using a data interval of 1 nm, was recorded. Secondly, the elapsed time associated with measuring the calibration curve and the sample (as described in Part 2) was also recorded.

Method

For both measurements, the signal averaging time was set at 0.1 s to ensure an equivalent analysis between the instruments. This setting is the shortest integration time possible for the Cary 8454 instrument.

A third timing measurement was done by repeating the two data collections on the Cary 3500 only. For this measurement the signal averaging time was set to 0.004 seconds. This is the shortest signal averaging time possible on the Cary 3500. The other parameters were unchanged.

Results

As shown in Table 4, the Cary 8454 has a fast processing time for measurement of a single spectrum. The benefit of this speed of analysis is clear for improved efficiency and throughput. The Cary 3500 presents a new paradigm for fast and efficient sample measurements by allowing multiple measurements simultaneously. The multicell module has no moving parts and measures each sample truly simultaneously. For analyses that require multiple measurements, such as calibration and measuring unknown samples, the Cary 3500 can preserve the integrity of the analysis, as no variations can arise between measuring standards and samples.

Table 4. Approximate elapsed time for measurements: comparison between the Cary 8454 and the Cary 3500 for a wavelength scan of a single sample, then for measurement of a calibration curve containing six standards.

	Cary 8454 (0.1 s integration time)	Cary 3500 (0.1 s signal averaging time)
Time for Wavelength Scan (s)	7	92
Time for Calibration Curve (s)	141	92

Conclusion

The Cary 8454 and the Cary 3500 UV-Vis spectrophotometers are both quick and easy to use. Both instruments have similar measurement capabilities as described here and produce equivalent results.

The extended linear range of the Cary 3500 compared to the Cary 8454 UV-Vis spectrophotometer was demonstrated. The ability to accurately measure highly absorbing samples, up to 4 Abs, extends the operational range of the instrument and removes errors associated with sample dilution.

The benefits of being able to measure multiple samples simultaneously was also demonstrated for the Cary 3500. Removing any environmental, experimental or instrument variables that can arise between standard and sample measurements enhances the quality of collected data.

There are many benefits for migrating methods from the Cary 8454 to the Cary 3500. Optional 21 CFR Part 11/EU Annex 11 compliance software tools are available for the Cary 3500. This option makes it an ideal replacement for the Cary 8454 in laboratories in GLP/GMP-regulated companies.

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