

# Comparison of Measurements performed on Cary 8454 and Cary 60 UV-Vis Spectrophotometers



## Introduction

Method development and validation is required to show all new instrumentation gives the same results within the operating range and acceptance criteria of existing instruments. Validation requirements when transferring a method from one instrument to another are dependent on GMP guidelines to ensure that results are comparable and reproducible.

This study demonstrates the transfer of a method between the Cary 8454 UV-visible spectrophotometer using UV-visible ChemStation software and the Cary 60 UV-visible spectrophotometer using Cary WinUV software. It also demonstrates that the results are accurate and reproducible when compared between the two spectrophotometer systems. Any further validation requirements for the method will need to meet regulatory guidelines and acceptance criteria.

## Experimental

### Equipment

- Cary 8454 UV-visible spectrophotometer
- UV-visible ChemStation software
- Cary 60 UV-visible spectrophotometer
- Cary WinUV software

### Reagents

- Blank solution: 0.00 mg/L perchloric acid blank
- Standard solutions: 40, 80, and 120 mg/L potassium dichromate
- Sample solution: approx. 75 mg/L potassium dichromate

### Part 1: Determining analysis wavelength

A sample of 40 mg/L potassium dichromate was used to determine an appropriate wavelength to use to generate a calibration curve using both the Cary 8454 UV-Vis and the Cary 60 UV-Vis.

#### Instrument parameters: Cary 8454

- ChemStation software: Standard Mode, Spectrum/Peaks Task
- Wavelength display: 200 - 800 nm
- Integration time: 0.5 seconds
- Peak/valley find: Find and annotate up to two peaks

#### Instrument parameters: Cary 60

- WinUV software: Scan application
- Wavelength display: 200 - 800 nm
- Scan speed: Advanced setup: data interval 3 nm
- Baseline: Correction selected
- Peak Information: 0.010 peak threshold, X label (Figure 1)

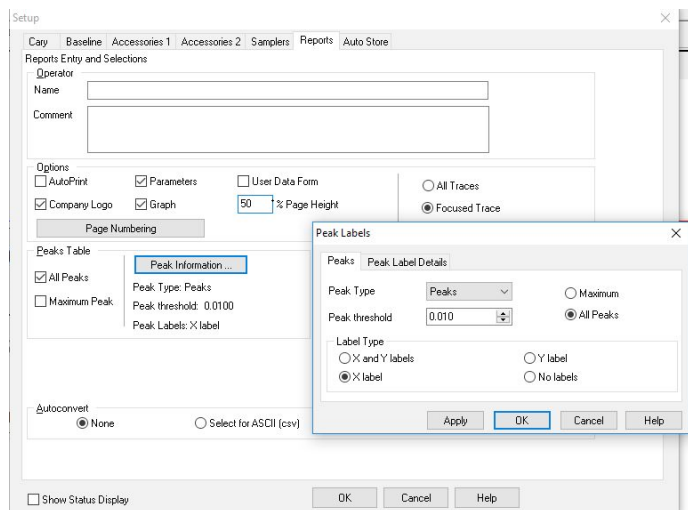


Figure 1. Setting up peak annotations in the Cary WinUV setup menu.

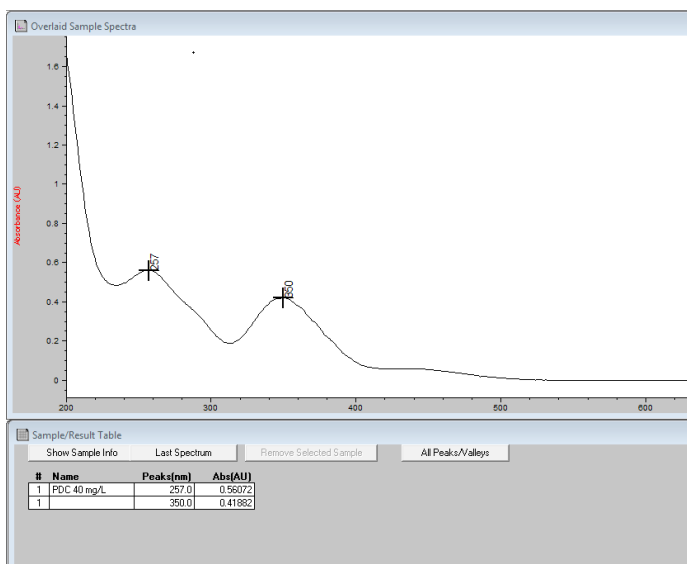
### Method

The Cary 8454 UV-visible spectrophotometer was switched on and lamps allowed to warm up for one hour. During the warm up the UV-visible ChemStation software was opened and 'Standard Mode' was chosen to perform the analysis and the parameters entered. After warm up, a blank reading was taken using the perchloric acid blank. A single spectra was collected using the 40 mg/L perchloric acid solution to identify the analysis wavelength.

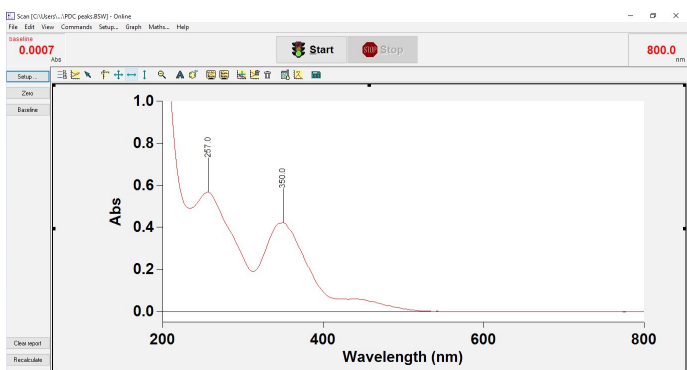
The Cary 60 UV-visible spectrophotometer does not require warm up. The Cary WinUV software was opened and 'Scan' application selected and parameters entered. A baseline was collected using the perchloric acid blank and then a scan of the 40 mg/L potassium dichromate solution was collected.

### Results

The potassium dichromate spectrum collected on both the Cary 8454 UV-vis and Cary 60 spectrophotometers demonstrate high reproducibility, with the same peaks identified at 257 nm and 350 nm (Figure 2a and 2b).



**Figure 2a.** UV-visible ChemStation software showing the potassium dichromate spectrum with two peaks identified at 257 nm and 350 nm. Collected on the Cary 8454 UV-vis spectrophotometer.



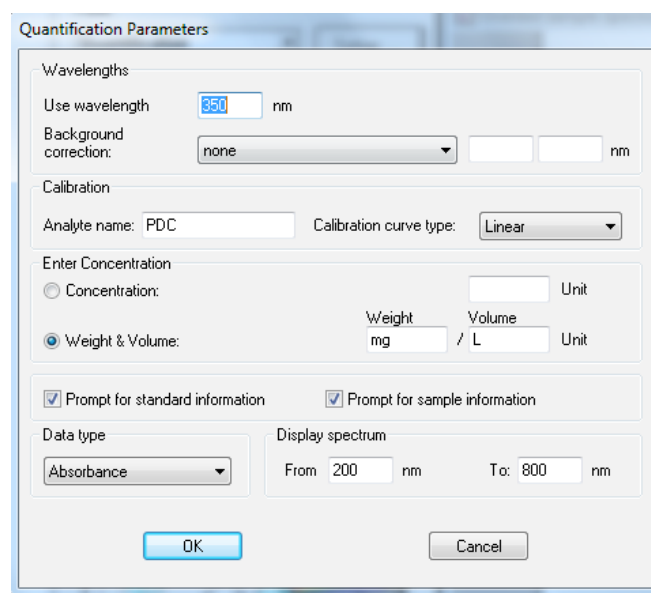
**Figure 2b.** Cary WinUV software showing the potassium dichromate spectrum with two peaks identified at 257 nm and 350 nm. Collected on the Cary 60 spectrophotometer.

## Part 2: Determining concentration of unknown sample

A calibration curve was generated using the Cary 8454 UV-vis and the Cary 60 using three standard solutions of potassium dichromate. The analysis wavelength was chosen to be 350 nm.

### Instrument parameters: Cary 8454 (Figure 3)

- ChemStation software: Standard Mode, Quantification Task
- Wavelength: 350 nm
- Background correction: none
- Calibration curve type: Linear
- Weight : mg/L units  
& Volume correction
- Prompts: for standard and sample information



**Figure 3.** Method parameters in the Quantification task of Standard Mode of UV-visible ChemStation

### Instrument parameters: Cary 60 (Figure 4)

- WinUV software: Concentration application
- Wavelength: 350 nm
- Standards setup: mg/L units, 3 standards, Linear fit
- Replicates: 2

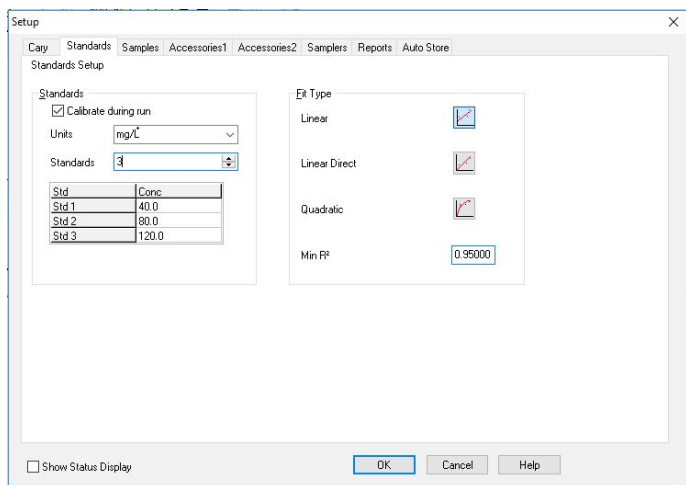


Figure 4. Method parameters in the Setup menu of the Concentration application of Cary WinUV

### Method

For both instruments, a blank reading was taken using the perchloric acid blank. A single measurement of the 40 mg/L, 80 mg/L and 120 mg/L perchloric acid solutions were taken to generate a calibration curve. The unknown sample was then measured.

### Results

A calibration curve was generated and the concentration of the unknown potassium dichromate solution was determined to be 77.5 mg/L by the Cary 8454 UV-vis (Figure 5) and 77.4 mg/L by the Cary 60 (Figure 6), demonstrating that the two systems can be used to generate equivalent results.

[www.agilent.com/chem](http://www.agilent.com/chem)

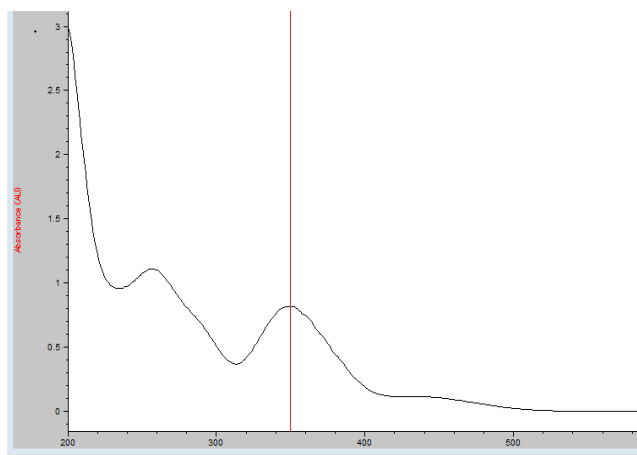


Figure 5. UV-visible ChemStation software showing the Sample/Result table with the concentration of the potassium dichromate unknown sample as 77.5 mg/L.

Analysis					
Collection time	7/3/2018 9:14:34 PM				
Sample	Concentration F mg/L	Mean	SD	%RSD	Readings
Sample 1					0.8225 0.8226
	77.4	0.8225	0.00007	0.00860	

Figure 6. Cary WinUV software Concentration application Analysis report showing the concentration of the potassium dichromate unknown sample (Sample 1) as 77.4 mg/L.

### Conclusion

The Cary 8454 UV-vis and the Cary 60 systems are both quick and easy to use. Both systems have software that permits easy method development, enabling quick setup of routine UV-Vis methods, such as those shown here.

The transfer of two methods from the Cary 8454 UV-visible spectrophotometer to the Cary 60 UV-visible spectrophotometer was demonstrated to be repeatable for peak picking and accurate for standard and sample measurements.

The demonstrated performance of the Cary 60 UV-visible spectrophotometer, which is also supported by optional 21 CFR Part 11 software tools, should give regulated laboratories confidence to use the Cary 60 with methods previously developed for an 8453 or 8454 instrument.

This information is subject to change without notice.