Seamless Method Transfer between the Agilent Cary 8454 UV-Visible Spectrophotometer and the 8453 UV-Visible Spectrophotometer

Technical note

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Introduction

A requirement of new instrumentation is that validated methods give acceptable and equivalent results as the instrument that the data was originally collected on. In regulated environments such as pharmaceutical laboratories, these methods typically comply with pharmacopeia regulations and requirements. By ensuring a seamless transition of methods from the 8453 UV-Vis spectrophotometer to the new Agilent Cary 8454 UV-Vis spectrophotometer, the aim is to eliminate the time consuming and costly need for revalidation.

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This application note demonstrates that the method used and results obtained using the Agilent 8454 UV-Visible spectrophotometer are accurate and reproducible when compared with data collected on the 8453 UV-Vis spectrophotometer. It also confirms that combined with UV-Visible ChemStation software, the Agilent Cary 8454 UV-Visible spectrophotometer system is the instrument of choice for routine quantitative and quality control applications in regulated laboratories.

Experimental

Equipment

- Agilent 8453 UV-Vis spectrophotometer
- Agilent Cary 8454 UV-Visible spectrophotometer
- UV-Visible ChemStation software

Instrument parameters

- UV-Vis ChemStation software: Advanced Mode
- Wavelength range: 190 nm – 1100 nm
- Integration time: 0.5 seconds
- Analytical wavelength: 297 nm
- Calibration curve: C = k1*A
- Calculation method: Least squares

Reagents

- Buffer solution: 0.02M acetate buffer
- Stock solution: 1 mg/mL salicylic acid in 0.02M acetate buffer
- Blank solution: 0.00 mg/mL salicylic acid in 0.02M acetate buffer
- Standard solutions: 0.00, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL salicylic acid
- Sample solution: approximately 0.02 mg/mL salicylic acid

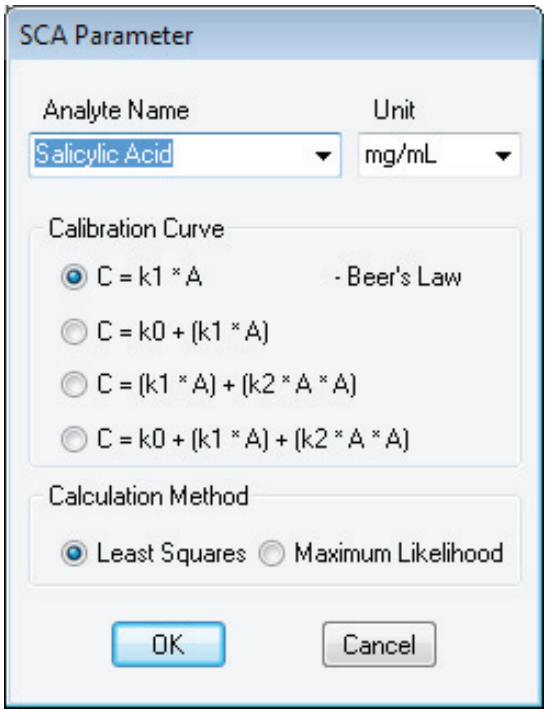

Figure 1. Instrument parameter settings dialog.

Method

Both the 8453 and 8454 UV-Vis spectrophotometers were switched on and the lamps allowed to warm up for one hour. During the warm up, the UV-Visible ChemStation software was opened and 'Advanced Mode' was chosen to perform the analysis. After warm up, a blank reading was taken. Three spectra for each standard were then collected. Triplicate scans of the sample solution were then measured and the concentration results were calculated.

Results

The salicylic acid standard spectra collected on both the 8454 and 8453 UV-Vis spectrophotometers demonstrate high reproducibility, with the standard curves generated at 297 nm showing an excellent correlation coefficient of 1.00 for each instrument (Figure 2a and 2b).

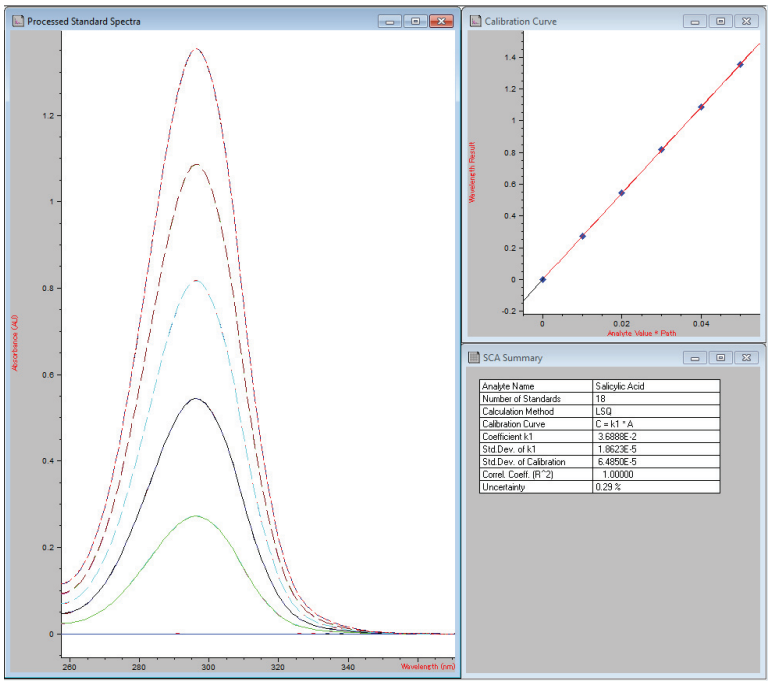

Figure 2a. UV-Visible ChemStation software showing triplicate salicylic acid standard spectra and resultant calibration curve at 297 nm; collected on the Agilent Cary 8454 UV-Visible spectrophotometer.

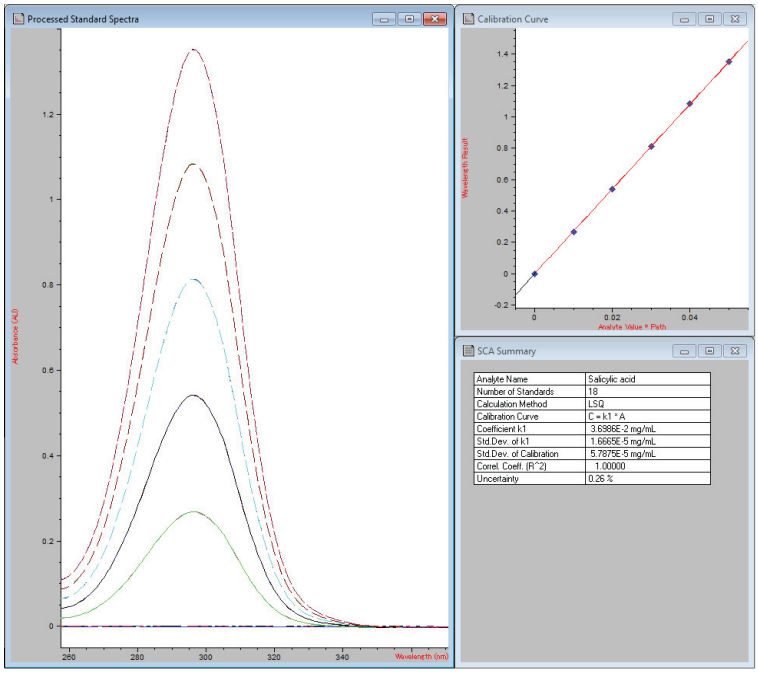

Figure 2b. UV-Visible ChemStation software showing triplicate salicylic acid standard spectra and resultant calibration curve at 297 nm; collected on the 8453 UV-Visible spectrophotometer.

The triplicate sample traces also showed very good reproducibility (Figure 3a and 3b).

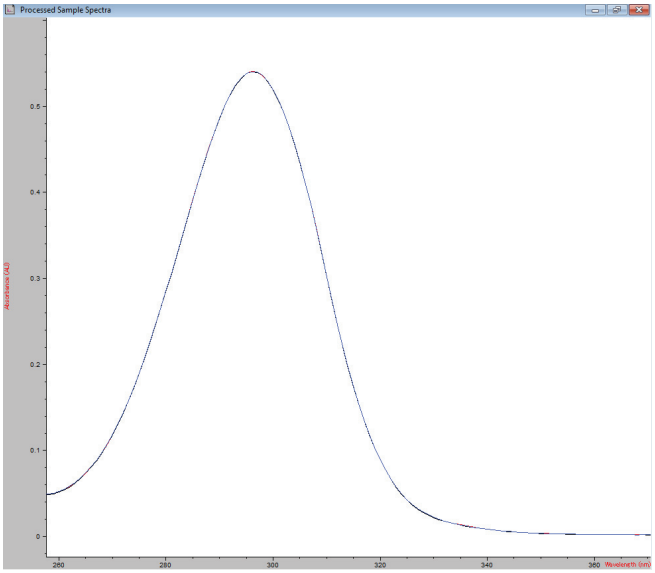

Figure 3a. UV-Visible ChemStation software showing triplicate sample spectra; collected on the Agilent Cary 8454 UV-Visble spectrophotometer.

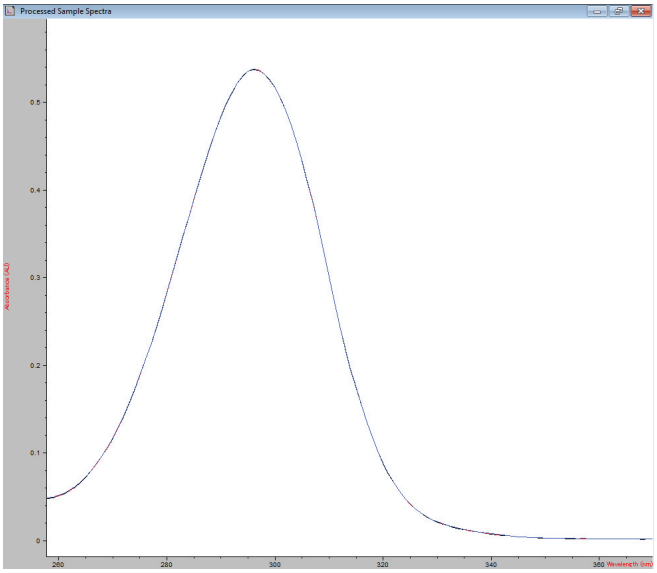

Figure 3b. UV-Visible ChemStation software showing triplicate sample spectra; collected on the 8453 UV-Vis spectrophotometer.

In addition to reproducible data being collected on each instrument, when the traces from each of the instruments are overlaid, the spectra also show excellent correlation, as seen in the overlay of the highest 0.05mg/mL salicylic acid standard (Figure 4).

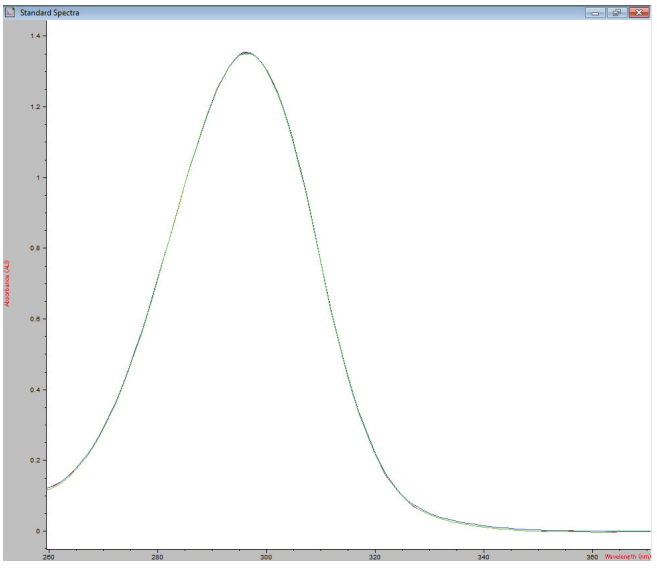

Figure 4. Overlay of 0.05 mg/mL salicylic acid standard spectra, triplicate traces collected on the 8454 and 8453 UV-Vis spectrophotometers. spectra; collected on the 8453 UV-Vis spectrophotometer.

Table 1. Average and standard deviation (SD) of triplicate scans for each standard and the sample.

	8454 instrument		8453 instrument		
	Average	SD	Average	SD	Difference from 8454
0.00mg/mL Salicylic Acid (Abs)	0.0000	0.0001	-0.0003	0.0001	-0.0003
0.01mg/mL Salicylic Acid (Abs)	0.2717	0.0002	0.2678	0.0002	-0.0039
0.02mg/mL Salicylic Acid (Abs)	0.5434	0.0002	0.5407	0.0003	-0.0027
0.03mg/mL Salicylic Acid (Abs)	0.8157	0.0003	0.8124	0.0002	-0.0033
0.04mg/mL Salicylic Acid (Abs)	1.0853	0.0001	1.0830	0.0003	-0.0023
0.05mg/mL Salicylic Acid (Abs)	1.3526	0.0004	1.3503	0.0005	-0.0022
Sample (Abs)	0.5397	0.0003	0.5368	0.0002	-0.0029
Sample concentration (mg/mL)	0.02	0.00	0.02	0.00	0.00

Detailed results (Table 1) confirm the reproducibility of the data collected at 297 nm, with very low standard deviations for the average of the triplicate standard and sample readings. The concentration measurements performed on both instruments successfully calculated the sample concentration as 0.02 mg/mL. The table also highlights the high reproducibility between the 8454 and 8453 instruments, with the average readings varying by no more than 0.0039 absorbance units, which did not show any effect of the end result.

Conclusion

The Agilent Cary 8454 UV-Visible spectrophotometer has the same performance specifications as the 8453 UV-Vis and is based on the proven technology of the diode array detector. This application note demonstrates the high reproducibility for standard and sample measurements achieved by the new Agilent Cary 8454 UV-Visible spectrophotometer. It also demonstrates the accuracy of the performed measurements, as highlighted by the 1.00 correlation coefficient for the salicylic acid standard curve.

The data collected on the Agilent Cary 8454 UV-Vis showed excellent reproducibility when compared with the results obtained using the 8453 UV-Vis spectrophotometer, varying by no more than 0.0039 absorbance units in this experiment, which is similar to the instrument to instrument reproducibility seen with 8453 instruments (data not shown).

The demonstrated performance of the Agilent Cary 8454 UV-Vis spectrophotometer should give regulated laboratories confidence to use the 8454, with methods previously developed for the 8453 UV-Vis spectrophotometer.

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