Application Note

Pharmaceuticals

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Improve Data Integrity and Remove Analytical Variables in UV-Vis Measurements

Cary 3500 Multicell UV-Vis spectrophotometer measures a standard curve and multiple samples simultaneously

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Introduction

Common quality assurance / quality control (QA/QC) processes in pharmaceutical environments require therapeutically active compounds and excipients to be regularly monitored during and following production. One well established approach is to determine the concentration via UV-Vis spectrophotometry using a calibration curve. Despite the reliability of this approach, there are some variabilities that may be introduced that can risk the data quality. These types of variables are most likely to originate from the instrument, environmental based errors, or operator errors.

Acetylsalicylic acid (ASA) is the active ingredient in Aspirin, a common anti-rheumatic, anti-inflammatory and general pain relief pharmaceutical agent (*1*). The compound was used to demonstrate an approach that is possible only with the Cary 3500 UV-Vis spectrophotometer. As with many common pharmaceuticals, a large percentage of the tablet consists of nonactive agents such as binders, color agents, solubilizing agents in addition to the active pharmaceutical ingredient (API).

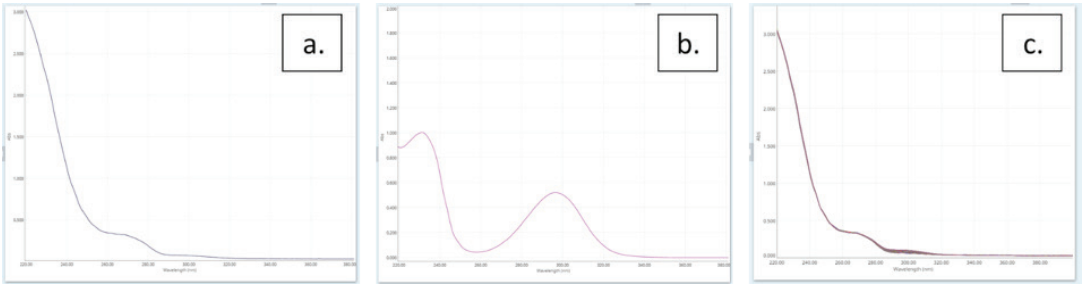

Figure 1. The absorbance spectra for (a.) ASA (b.) SA and (c.) the absorbance signal of the ASA sample after four hours at 25 °C showing the hydrolysis reaction proceeding.

The percentage composition of ASA can readily be determined by hydrolyzing the ASA to its de-acetylated form, salicylic acid (SA). The SA is then quantified by measuring the UV-Vis absorbance and referring to a calibration curve.

Environmental based errors can come from many sources, but may include a marked change in absorbance signal with only a small change of temperature or contact with water. ASA is highly unstable when in contact with water and will proceed to hydrolyze to SA even at room temperature (Figure 1), resulting in a vastly different absorbance profile.

This instability means that analysis using conventional UV-Vis spectrophotometers must be performed very quickly, under specific pH conditions, and at specific temperatures. Alternatively, the ASA could be converted to SA before determining the concentration. This approach assumes that there is no free SA present initially, which has been previously shown not to be the case (2). The hydrolysis can be a significant convoluting factor with increasing temperature, as shown in Figure 2. This suggests that minimizing potential environmental errors should be an important consideration for this sample.

Many experimental designs include performing analysis replicates as a means of avoiding operator error. Other sources of error may come from intended and unintended biases in sample preparation, or accidents that can happen between sample measurements leading to errors in measurements. The Cary 3500 Multicell allows simultaneous measurement of eight cuvette positions. A total of seven standards and samples (and one reference) can be analyzed at exactly the same time, under exactly the same conditions. The Cary 3500 multicell provides a new way to achieve data integrity by measuring samples, standards, and controls simultaneously. This capability eliminates environmental and operator-generated analytical variables and the resultant risk to data accuracy.

The Cary 3500 also has some key features that minimize instrument-introduced variability. The instrument has no moving parts requiring alignment, preventing errors due to incorrectly aligned sample holders. The instrument can also measure samples and standards simultaneously. This capability prevents errors due to changes in instrument performance and calibrations over time. In this way, the Cary 3500 helps minimize the variability that can be introduced via instrumental errors.

In this application note, the percentage composition of a commercially available aspirin sample was determined. The analysis was done via a base hydrolysis analysis, followed by absorbance measurements at 296 nm. The measurement of six standards and one sample simultaneously allowed possible environmental, operator, and instrument sources of error to be minimized.

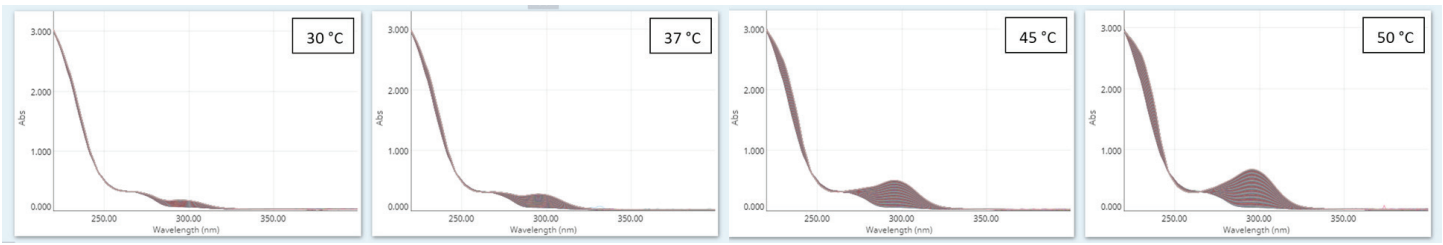

Figure 2. The conversion of ASA to SA at 30, 37, 45 and 50 °C over a four-hour period highlighting the increased reaction rate with temperature.

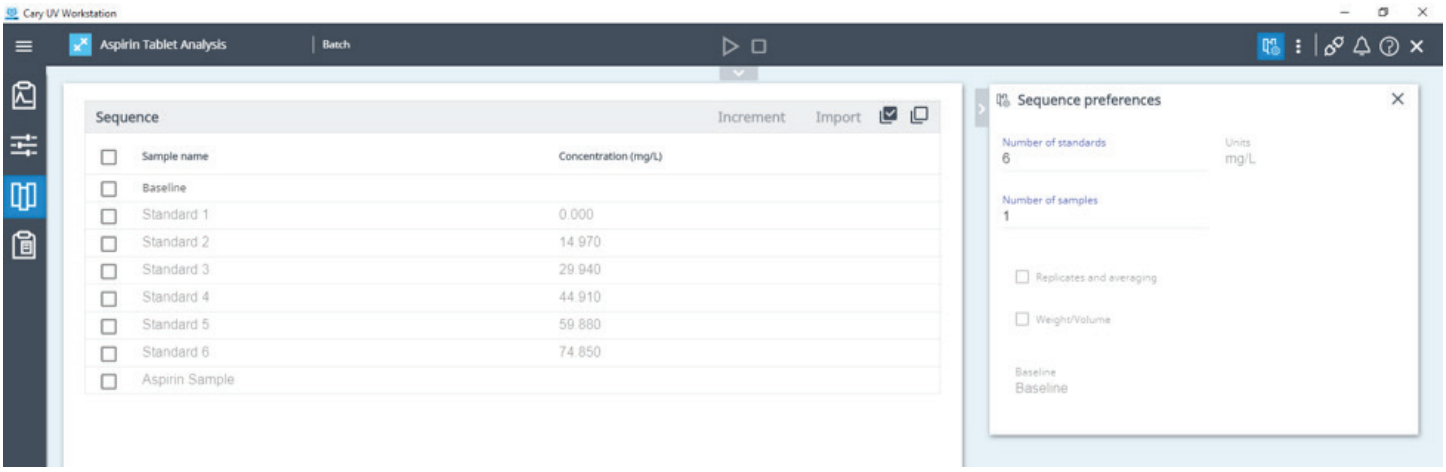

Figure 3. Sequence page in Cary UV Workstation Concentration application, showing the baseline selection, the number of standards and samples, along with unit selection and standard concentrations.

Experimental

Standard solution preparation

A standard curve of SA was prepared by dissolving 0.2 grams of neat SA in phosphate buffer solution (PBS) prepared in Milli-Q filtered water and set to a pH of 7.5. The standard curve was prepared as shown in Table 1 allowing an absorbance range of approximately 0 to 2 absorbance units to be established.

Table 1. Concentrations of prepared standards.

Standard ID	Concentration (mg/L)
Standard 1	0
Standard 2	14.97
Standard 3	29.94
Standard 4	44.91
Standard 5	59.88
Standard 6	74.85

Commercial Aspirin tablet base hydrolysis

A commercially available aspirin tablet sample was purchased. The tablet weighed 163.7 mg. It was dissolved in Milli-Q filtered water at 75 °C. The pH was set to approximately 12 with 1 M sodium hydroxide. The sample was held under these conditions for 1 hour to allow complete hydrolysis of the ASA acid to SA.

The sample was made up to 1 L, and then diluted to fall within the calibration range, and adjusted to a pH of 7.5.

Instrumentation

The Cary 3500 Multicell UV-Vis spectrophotometer was selected for this study due to its ability to measure eight cuvettes at the same time. This capability ensures measurement of each under the same conditions.

Table 2. Instrument parameters.

Parameter	Setting
Wavelength Range (nm)	400 to 250
Spectral Bandwidth (nm)	2
Signal Averaging Time (s)	0.4
Data Interval (nm)	1
Slice Wavelength (nm)	296
Zone Configuration	1 Zone

To perform the scans, 2.5 mL of each standard and sample was transferred into a 3.5 mL quartz cuvette, along with a Milli-Q water reference. Using the Concentration application in the Cary UV Workstation software, a single zone was selected to allow all seven positions to be measured simultaneously with a single reference. The parameters outlined in Table 2 were used.

The concentrations of the prepared standards were entered, and the baseline was applied (Figure 3). A total of six standards were selected to build the calibration curve, along with the aspirin tablet sample.

The baselines were collected for each cuvette position. Standards and samples were then measured in a single simultaneous measurement.

Results and discussion

Simultaneous analysis of standard curve and tablet samples

The wavelength scan was performed from 400 to 250 nm, allowing qualitative and quantitative analysis of the SA peak. The peak maximum was determined to be positioned at 296 nm. All standards and the sample were analyzed simultaneously using the Cary 3500 Multicell. This means that all standards and samples were measured at the same time, and therefore under the exact same conditions.

Following the wavelength scan of the six standards and the sample, the absorbance reading at 296 nm was determined for each. From this data, a standard curve was created, and the concentration of the tablet sample was calculated. The concentration of SA in the aspirin tablet sample was determined to be 7.755 mg/L (77.55 mg/L when accounting for dilution factor). This concentration corresponds to 101.15 mg of ASA in the tablet (Figure 4).

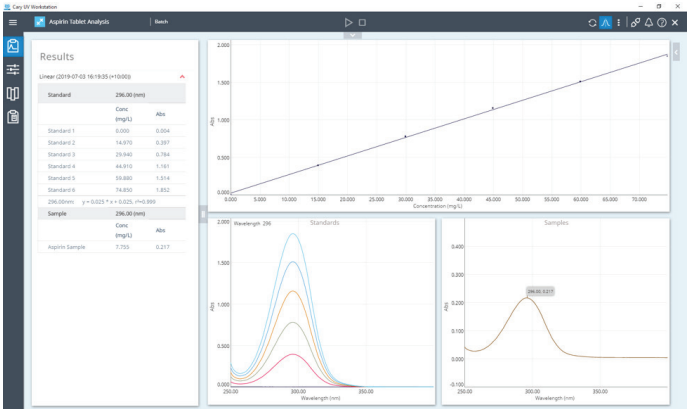

Figure 4. The Cary UV Workstation software showing the SA standard curve (top middle) along with the associated scans for the standards (bottom center) and the tablet sample (bottom right). On the left, the linear equation and the correlation coefficient can be seen along with the raw values at 296 nm.

From the determined mass of the sample tablet, the percentage composition can be calculated. The mass of the entire low dose aspirin tablet was 163.7 mg. The mass of ASA in the tablet was determined to be 101.15 mg, or 61.70 % of the tablet composition.

Conclusion

A commercially available aspirin tablet was purchased and subjected to a base hydrolysis reaction. From the resultant solution, the concentration of SA, and therefore ASA was determined. A standard curve of SA was prepared, and all standards and samples were analyzed under simultaneous conditions. This approach removed the risk of environmental, instrument, or operator error influencing the result obtained. The mass of ASA in the tablet was determined to be 101.15 mg, or 61.70 % of the tablet composition.

The Cary 3500 multicell provides a new way to achieve data integrity by measuring samples, standards, and controls simultaneously. This capability eliminates environmental and operator-generated analytical variables and the resultant risk to data accuracy.

References

1. Erkan D, Harrison MJ., Levy R., Peterson M., Petri M., Sammiriatno L., Unalp-Arida A., Vilela Y., Yazici Y., Lockshin MD. Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: a randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals, *Arthritis Rheum*, **2007**, 7, 2382-91.
2. Wang Y., Xu P., Li X., Nie K., Tuo M., Kong B., Chen J. Monitoring the hydrolyzation of aspirin during the dissolution testing for aspirin delayed-release tablets with a fiber-optic dissolution system, *J Pharm Analysis*, **2012**, 2, 386-389.

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