Analysis of Two Active Pharmaceutical Ingredients (API) Products Using UV Spectrophotometry with Multi-Component Analysis and a Fiber Optic Dissolution Analyzer

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Introduction

UV Spectrophotometry has traditionally been a simpler and less time and labor consuming method for analyzing dissolution testing samples. However, as soon as a product contained more than one active pharmaceutical ingredient (API), analysis with UV was no longer considered an option. This is because both species often absorb over the same spectral region, causing deviations from Beer-Lambert Law. This linear relation between absorbance and the absorbing species is the basis for calculating concentration values based on the measured absorbance at a specific wavelength. In these cases, separation techniques such as HPLC become the de facto analysis methods.

While it has been long shown that using Multicomponent Analysis (MCA) software and complete spectral and temporal profiles make it possible to analyze such products using UV, the drawback has always been the difficulty in acquiring all the required data. However, with a modern fiber optic UV dissolution analyzer, these obstacles have been removed. Analysis of the two spectrally overlapping components is accomplished by applying the Classical Least Squares form of Multiple Linear Regression to the complete spectral and temporal profiles obtained using these new analyzers. The algorithm uses a calibration matrix of extinction coefficients to calculate component concentrations in an unknown mixture. These are derived from a training set comprised of the spectra of multiple standard solutions.

This white paper explains the theory behind the MCA algorithm methodology. Then, used in tandem with in-situ fiber optics, the accuracy of the technique is demonstrated by recovering the concentration of two APIs in known mixed solutions. Finally, an example is given of accurately monitoring and quantifying the dissolution profile of an actual commercial product containing two APIs, demonstrating the elimination of the need to draw samples or to perform HPLC analysis for many of these type of products.

In the case of dissolution, Classical Least Squares analysis involves the application of Multiple Linear Regression to the classical expression of the Beer's law. Since complete UV spectra are measured, Beer's law can be expanded to incorporate absorbance of multiple components at different wavelengths, λ:

$$A_\lambda = \sum_{j=1}^{p} E_{\lambda j} \cdot c_j \qquad (1)$$

Where:

A_λ = Absorbance of the mixture of p components at wavelength λ

$E_{\lambda j}$ = Response sensitivity factor (molar absorptivity × probe path length) of component j at wavelength λ

Cj = Concentration of component j in the mixture

However, interactions between components including excipient materials also need to adequately represented. This leads to the need to expand the simple equation above into a more complex matrix:

$$A = K \cdot C \qquad (2)$$

Where:

A = Matrix of absorbance values for the calibration solutions

K = Matrix of sensitivity factors determined from measured spectra of mixtures with known component concentrations.

C = Matrix of known standard concentration values

K is calculated using the concentration matrix C, its transpose C^T, and the calibration set absorbance matrix A_{std}.

$$K = A_{std} \cdot C^T \cdot [C \cdot C^T]^{-1} \quad (3)$$

From K and its transpose K^T, K_{cal} (referred to as the calibration or regression matrix) can then be generated:

$$K_{cal} = [K^T \cdot K]^{-1} \cdot K^T \quad (4)$$

The least-squares solution to determining analyte concentrations in an unknown mixture is then determined by the applying K_{cal} to the measured absorbance values of the unknown mixture A_{unk},

$$C_{unk} = A_{unk} \cdot K_{cal} \quad (5)$$

C_{unk} is the vector containing predicted concentration values (C_1, C_2, …, C_n) for each analyte in the unknown mixture.

As an example of the ability of this technique to measure the concentrations of components in a mixed solution, known mixtures of two ingredients found in common OTC products, Acetaminophen and Caffeine were measured.

The spectra of pure standards of Acetaminophen and Caffeine are shown in Figure 1.

The technique was then used to analyze data collected using the Distek Opt-Diss 410 Fiber Optic Dissolution System from five mixtures with varying amounts of Acetaminophen and Caffeine. The computed values produced by the Opt-Diss 410 MCA software are compared to the actual values in the Table 1 and represented graphically in Figure 2.

One can clearly see that the method accurately quantitates the amounts of Acetaminophen and Caffeine in mixtures with an error well less than 2%.

To illustrate the applicability of the technique to real measurements, the dissolution of a tablet containing 400 mg Aspirin and 32 mg

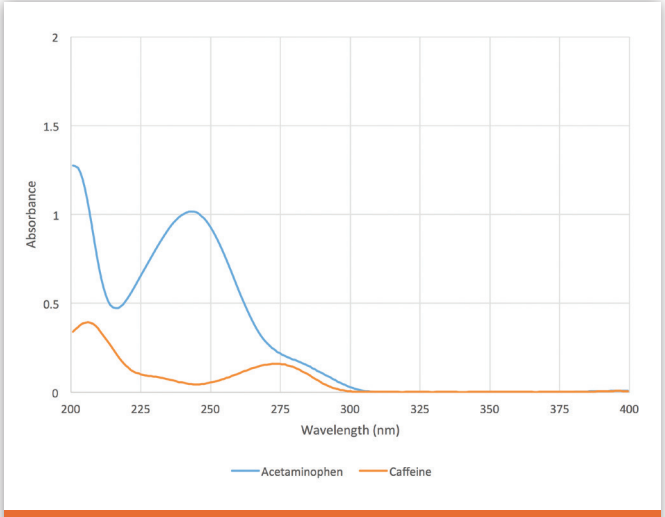

Figure 1. Absorbance spectra of Acetaminophen and Caffeine standards.

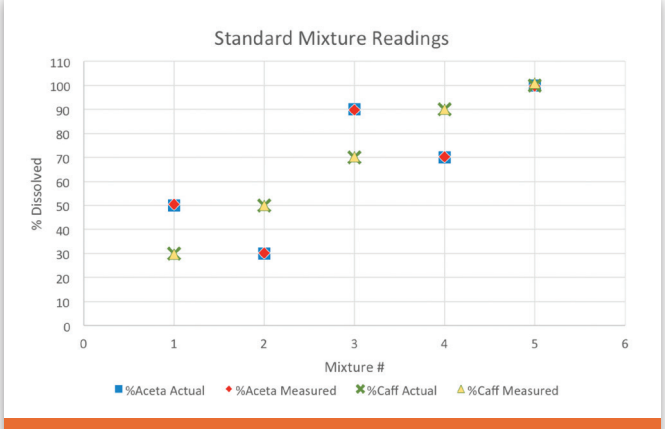

Figure 2. Comparison of measured versus actual results of standard mixtures.

Caffeine was analyzed. Absorbance spectra of pure standards of Aspirin and Caffeine at 80% are shown in Figure 3.

Complete spectra from all six vessels were collected every 10 seconds for 30 minutes, again using the Distek Opt-Diss 410 Fiber Optic

Table 1. Measured versus actual percentage values of standard mixtures.

	Acetaminophen			Caffeine		
Mixture	**% Actual**	**% Measured**	**% Error**	**% Actual**	**% Measured**	**% Error**
1	50	50.43	0.9%	30	29.60	1.3%
2	30	30.30	1.0%	50	50.08	0.2%
3	90	89.83	0.2%	70	70.40	0.6%
4	70	70.19	0.3%	90	90.16	0.2%
5	100	99.68	0.3%	100	100.96	1.0%

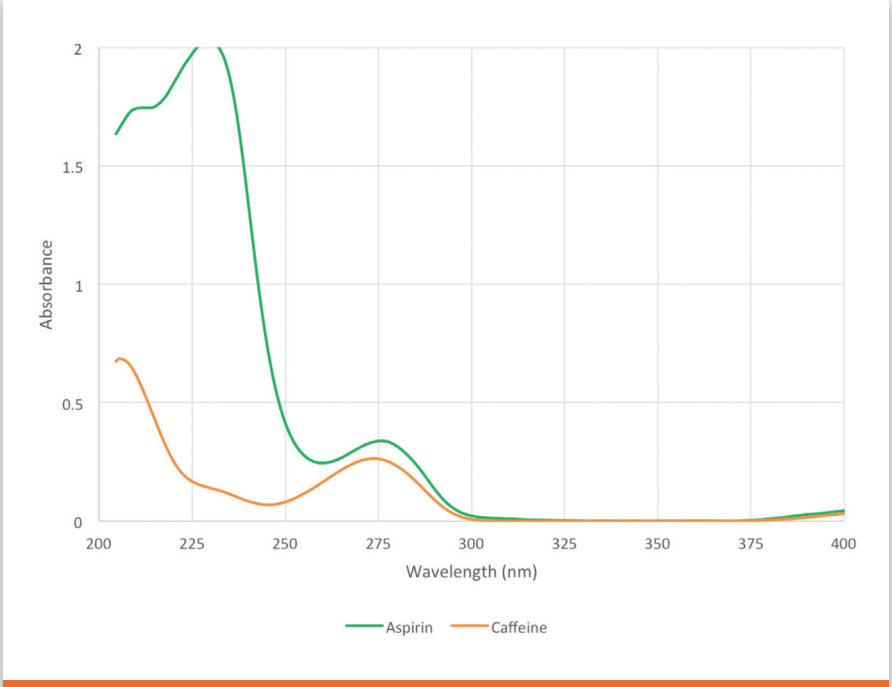

Figure 3. Absorbance spectra of Aspirin and Caffeine standards.

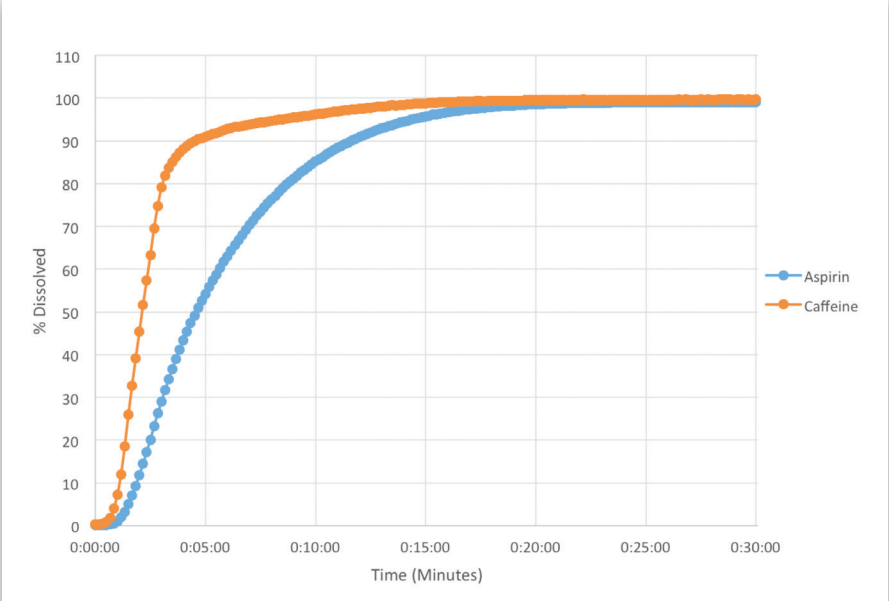

Figure 4. Average dissolution profile of six Aspirin Caffeine tablets.

Dissolution System. The results were then analyzed using the method described above. In this case, the training set used comprised the measured values of five different mixtures of Aspirin and Caffeine plus the 80% pure standards shown in Figure 3.

As the results in Figure 4 demonstrate, the technique measures the simultaneous dissolution rates of the two components, readily resolving caffeine's very fast release rate as well as aspirin's slower one.

Summary

UV spectrophotometry combined with MCA has been demonstrated to yield accurate analysis of the absolute concentrations of each component in two component mixtures. The technique has been also successfully applied to measuring the separate dissolution rates of two APIs in a commercially available product. These results demonstrate the method can accurately quantify two components even with highly overlapping spectra without the need for a separation step. The key to this process is using large data sets consisting of large spectral regions instead of individual wavelengths and complete temporal profiles instead of a few points. This rich data set collection is enabled by the use of in-situ sampling utilizing fiber optics probes which analyze the sample within the vessel. This circumvents the limit of the speed of moving the liquid from vessel to the analyzer that encumber traditional methods such as HPLC or conventional UV spectroscopy. An additional benefit of the instantaneous data collection of in-situ probes is that they allow near real-time dissolution analysis.

As these measurements of commercial products under real-world conditions illustrate, the addition of MCA and fiber optic in situ measurements allow formulation and analytical chemists, as well as QC analysts to realize the time and labor savings associate with UV spectrophotometry even when measuring products with two APIs.

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