

UV/Visible Spectroscopy

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Pharmaceutical Assay and Multicomponent Analysis using the LAMBDA 365+ UV/Vis Spectrophotometer

Both active ingredients have a similar mode of action, whereby they inhibit the cyclooxygenase (COX) enzyme, by preventing the production of prostaglandins which cause pain, inflammation, and fever. UV/Vis spectrometry is a fast and commonly used technique in quality control laboratories for routine analysis of purity and quantity of components within various stages of a product's manufacture in many industries.

Introduction

Combination drugs containing Paracetamol and Aspirin, displayed in Figure 1, are widely used analgesics with anti-inflammatory properties for treatment of migraines.

Title 21 of the Code of Federal Regulations (CFR), which establishes the Food and Drug Administration (FDA) regulations to ensure traceability, requires FDA-regulated industries to keep electronic records, audits, and documentation for systems used, and also to perform installation, operational, and performance qualification (IQ/OQ/PQ).¹ This application describes the efficient use of the LAMBDA® 365+ in determining the quantity and percent assay of Paracetamol according to U.S. Pharmacopeia's (USP) methods, and component concentration in pharmaceutical formulations, while achieving 21 CFR Part 11 compliance.

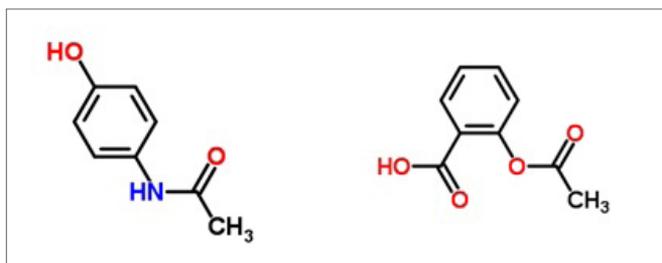


Figure 1. Chemical structures of Paracetamol (left) and Aspirin (right).²

Principle

Single component pharmaceutical assays can be determined using single-point standardization, which involves measuring the absorbance spectrum of a standard solution of the reference sample and a sample solution. The Beer-Lambert law, as shown in Equation 1, states that the absorbance of a particular species in a sample is directly proportional to the concentration of the absorbing species, providing the pathlength of the sample remains constant. As a result, the concentration of the single component in the sample can be determined from this relationship.

Equation 1

Beer-Lambert law (A – absorbance, ϵ – molar extinction coefficient, c – concentration, l – pathlength)

$$A = \epsilon \cdot c \cdot l$$

This proportionality remains valid for multicomponent samples that contain more than one absorbing species. In a mixture, the absorbance at a particular wavelength is equal to the sum of the individual absorbance contributions from each absorbing species at that wavelength. As a result, for assay of substances in multicomponent samples, a simultaneous equation method can be used to determine the concentration of each component as shown below for a binary mixture (x and y).³

$$A'_{(x+y)} = A'_x + A'_y = \epsilon'_x c_x l + \epsilon'_y c_y l$$

$$A''_{(x+y)} = A''_x + A''_y = \epsilon''_x c_x l + \epsilon''_y c_y l$$

Where:

A' - absorbance at wavelength'

A'' - absorbance at wavelength''

ϵ' - molar extinction coefficient at wavelength'

ϵ'' - molar extinction coefficient at wavelength''

c - concentration

l - pathlength of sample

Paracetamol Assay

Method

Paracetamol powder meeting USP specifications was obtained from Sigma-Aldrich (A5000). A stock solution of Paracetamol (100 mg/L) was prepared in a 100 mL volumetric flask by dissolving Paracetamol powder (10 mg) in 1 mL of methanol and diluting to the total volume with deionised (DI) water. A working Paracetamol standard in 100 mL was prepared by taking 10 ml of the stock solution and using DI water as the diluent. A test sample was also prepared using the same method as the working standard.⁴

Using UV Express™ software and the LAMBDA 365+, the spectra of the working standard and test sample were scanned between 200 - 400 nm, using DI water as the blank. The absorbance of these samples was measured at the wavelength of maximum absorption (λ_{max}) of 243 nm using the instrument parameters shown in Figure 2.

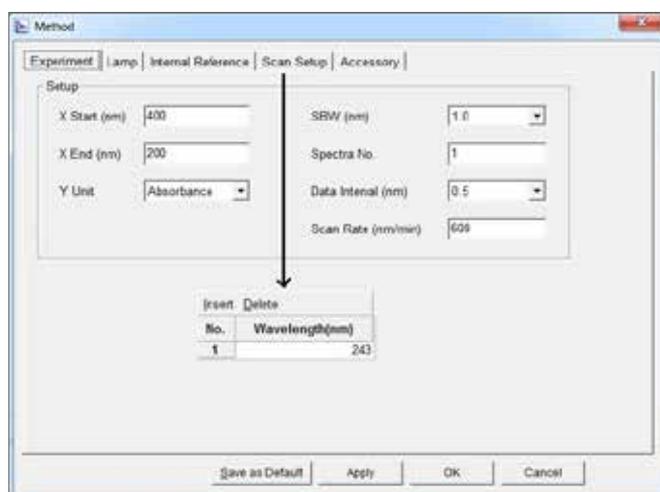


Figure 2. LAMBDA 365+ instrument parameters and wavelength monitoring setup.

Results

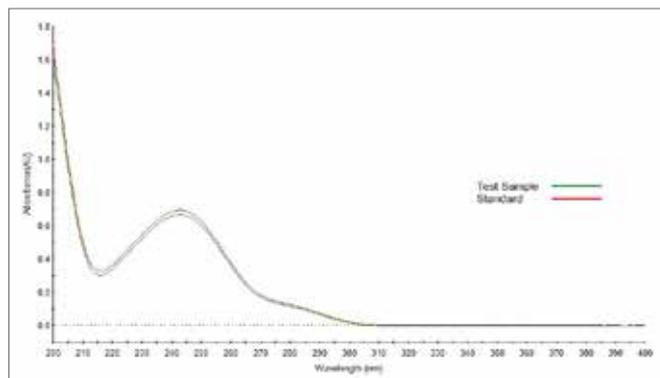


Figure 3. Spectrum scan of standard and test sample.

Figure 3 shows spectra of the Paracetamol standard and test sample, where the absorbance at 243 nm was determined to be 0.693 and 0.667 respectively. The quantity and percent assay of Paracetamol in the test sample was calculated using equations in 2a and 2b.

Equation 2a.

$$\begin{aligned} \text{Paracetamol in test sample (mg)} &= \\ \text{Standard sample weight (mg)} \times \frac{A_{243} (\text{Test sample})}{A_{243} (\text{Standard sample})} &= \\ = 10.5 \text{ mg} \times \frac{0.667}{0.693} &= \\ \text{Paracetamol in test sample} &= \mathbf{10.1 \text{ mg}} \end{aligned}$$

Equation 2b.

$$\begin{aligned} \text{Paracetamol percent assay in test sample} &= \\ \frac{\text{Paracetamol percent assay in test sample (mg)}}{\text{Weight of test sample (mg)}} \times 100 &= \\ = \frac{10.1 \text{ mg}}{10.3 \text{ mg}} \times 100 &= \\ \text{Paracetamol percent assay in test sample} &= \mathbf{98.1 \%} \end{aligned}$$

The quantity of Paracetamol in the test sample was determined to be 10.1 mg and, using this calculated value, the percent assay was determined to be 98.1%. USP 29 states a test sample should contain “not less than 98.0 percent and not more than 101.0 percent C₈H₉NO₂, calculated on the anhydrous basis.”⁵ The Paracetamol test sample was found to be within this range and thus in accordance with USP methods.

Multicomponent Analysis

Method

Two individual stock solutions of 100 mg/L Paracetamol and 100 mg/L Aspirin were prepared in 100 ml volumetric flasks by dissolving each powder (10 mg) using 0.1 M HCl as the diluent and sonicating for 20 minutes. From these stock solutions, 10 mg/L Paracetamol and Aspirin working standards were prepared in 100 ml volumetric flasks by dilution with 0.1 M HCl.

Using the Multicomponent Analysis (MCA) mode in the UV Express software, the LAMBDA 365+ scan parameters were set to measure the wavelength range 200 - 400 nm. The two pure 10 mg/L Paracetamol and Aspirin solutions were measured as standards, Figure 4, in 10 mm pathlength quartz cuvettes, using 0.1 M HCl as the blank. Three sample solutions were prepared in 4 mL, using the pure Paracetamol and Aspirin solutions in varying proportions, shown in Table 1, to create two-component mixture samples. The absorbance spectrum of each sample was measured to determine the concentration of each component in the mixtures.



Figure 4. Multicomponent analysis setup.

Table 1. Concentrations for 2-component mixtures.

Sample	Concentration of Paracetamol (mg/L)	Concentration of Aspirin (mg/L)
1	7.50	2.50
2	5.00	5.00
3	2.50	7.50

Results

Overlaid absorbance spectra of the pure Paracetamol and Aspirin standards are shown in Figure 5 and sample mixture spectra shown in Figure 6. Table 2 displays the calculated concentrations, using the software's simultaneous equation algorithm, in comparison with the expected ratios. The calculated concentrations of Paracetamol and Aspirin in sample 2 are 4.82 mg/L and 5.03 mg/L respectively, close to the 5:5 expected ratio. The results show a good level of accuracy over the component concentration ranges.

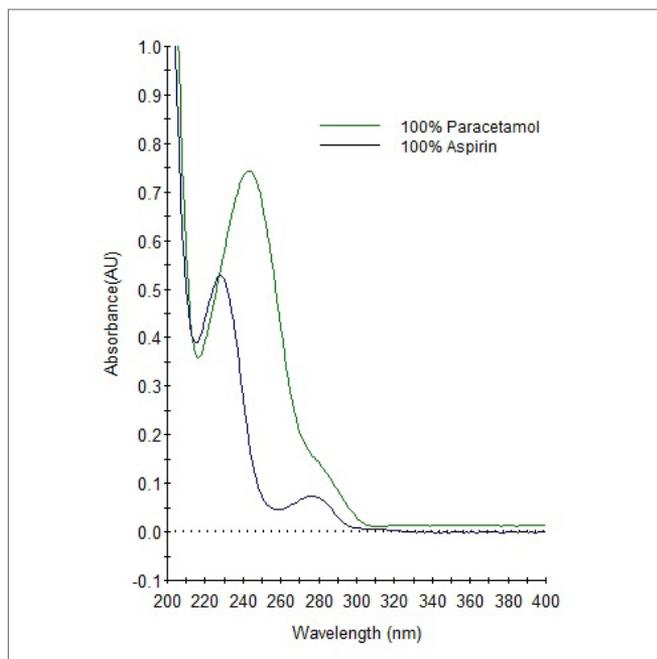


Figure 5. Absorbance spectra of Paracetamol ($\lambda_{\text{max}} = 243 \text{ nm}$) and Aspirin ($\lambda_{\text{max}} = 228 \text{ nm}$) standards.

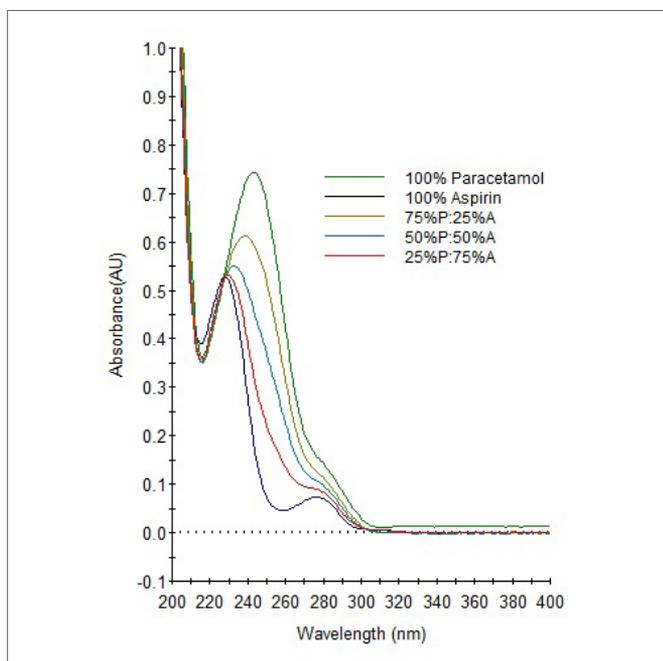


Figure 6. Overlaid standard and multicomponent sample spectra.

Table 2. Calculated Paracetamol and Aspirin component concentrations.

Sample	Expected Ratio (Paracetamol: Aspirin)	Calculated Paracetamol Concentration (mg/L)	Calculated Aspirin Concentration (mg/L)
1	7.5 : 2.5	7.25	2.58
2	5.0 : 5.0	4.82	5.03
3	2.5 : 7.5	2.42	7.33

Conclusion

In this application, the percent Paracetamol assay was determined to be in accordance with the USP 29 assay test for Paracetamol, and multicomponent analysis of pharmaceutical mixtures was achieved with a high level of accuracy. The LAMBDA 365+ with UV Express software enables rapid and easy determination of pharmaceutical assays, and accurate quantitative determination of multicomponent mixtures, while achieving 21 CFR Part 11 compliance. Additional software features enable the LAMBDA 365+ to support a full range of applications and methods to those requiring regulatory compliance.

References

1. U.S. Department of Health and Human Services Food and Drug Administration, 'Guidance for Industry Part 11, Electronic Records; Electronic Signatures — Scope and Application'.
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5. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m200.html.