Application note

Quality Control of Small Molecule Pharmaceuticals Using Spectroscopy

Introduction

The quality control of pharmaceutical products is essential to ensure their safe and effective use by patients. The Thermo Scientific™ NanoDrop™ Eight 8-channel Microvolume UV-Vis Spectrophotometer offers a rapid and cost-effective method for spot-checking batch quality at various points in the pharmaceutical production line. The concentrations of many compounds can be determined based upon UV-Visible (UV-Vis) absorbance, and purity can also be monitored by analyzing spectral data.

The auto-ranging pathlength technology of the NanoDrop Eight spectrophotometer enables quantification of pharmaceutical samples across a much broader concentration range than is possible with a conventional cuvette-based spectrophotometer. By automatically selecting the optimum pathlength (ranging from 1.0 mm to 0.1 mm), the NanoDrop Eight spectrophotometer can accurately measure the absorbance of a sample across a dynamic range nearly 200-fold greater than that of a cuvette-based system.

Experimental Procedures

Per the US Pharmacopeia specifications, unknown concentrations of acetaminophen must be quantified using absorbance at 244 nm against an acetaminophen standard curve prepared from a known standard of ≥99.9% purity.[1] Prior to experimentation, the absorbance maximum of acetaminophen was confirmed as being at 244 nm (Figure 1).

A 3.0 mg/mL standard was prepared using 30 mg acetaminophen (Sigma-Aldrich, A7085) dissolved in 10 mL deionized H_2O. A standard curve consisting of 7 standards was prepared by performing serial dilutions to yield a concentration range of 3.0 mg/mL – 0.09 mg/mL plus a 0.0 mg/mL control. Six unknown acetaminophen samples were measured against the standard curve to experimentally determine concentrations and calculate the total acetaminophen content of each sample. The spectrophotometer was blanked with deionized H_2O prior to making measurements.

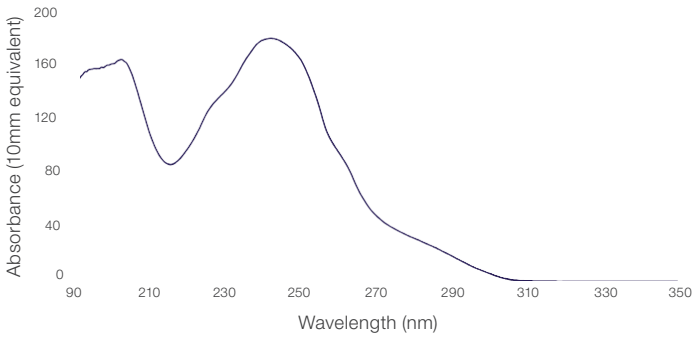

Figure 1. Absorbance spectrum of the 3.0 mg/mL acetaminophen standard. Absorbance maximum at 244 nm was used for subsequent analysis.

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A Custom Method – Standard Curve program was created in the NanoDrop Eight software to enable concentration determination of unknown samples against the standard curve without the need for calculations outside of the software. The measurement range of the custom method was set to the UV range of 190–350 nm to accommodate the 244 nm Analysis Wavelength of acetaminophen. Baseline Correction was set to 340 nm to adjust the baseline from background interference. Finally, Automated Pathlength was turned on to ensure the correct pathlength would be selected based on the absorbance intensity at the Analysis Wavelength of 244 nm. This option prevents detector saturation if the absorbance intensity is above 12.5A.

Results

A strong linear relationship between absorbance and concentration was observed throughout the entire standard curve range (Figure 2). The R^2 of 0.9997 confirms an accurate prediction of concentration based on the absorbance at 244 nm.

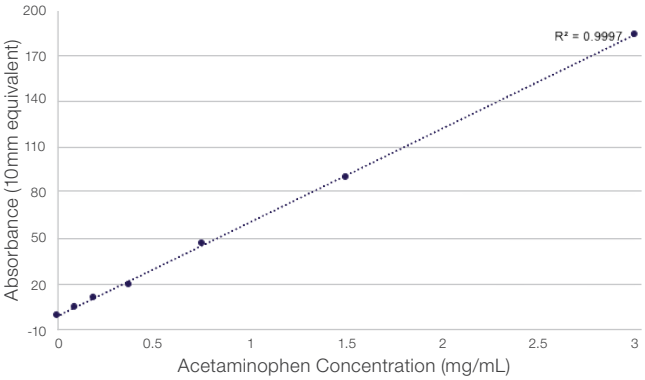

Figure 2. Standard curve of acetaminophen absorbance at 244 nm.

The six unknown acetaminophen samples were measured in triplicate to assess reproducibility (Table 1). The percent coefficient of variation (%CV) was below 1.5% for all six samples, indicating excellent reproducibility between replicate measurements.

Conclusion

The linear relationship between acetaminophen concentration and absorbance at 244 nm makes spectrophotometry an ideal method to verify batch concentrations. The NanoDrop Eight spectrophotometer can be employed to provide a rapid and accurate spot-check of pharmaceutical products. The instrument's auto-ranging pathlength technology greatly reduces the need for sample dilution, which often causes costly errors and delays. When utilizing a traditional cuvette-based system, a greater number of large volume serial dilutions would be required to dilute the samples from their stock concentrations to a measurable concentration range. By reducing or completely eliminating the number of dilutions required, the NanoDrop Eight instrument greatly reduces this potential source of error.

In addition, the full spectrum display makes the NanoDrop Eight instrument ideal for providing insight into the purity of various batches of product. The short measurement cycle and general ease of use also greatly increase the rate at which batches can be processed, making it possible to implement multiple quality control checks throughout the production process.

Sample	Replicate	A244	Concentration (mg/mL)	%CV
A	1	1.943	118.338	0.813
	2	1.96	119.389	
	3	1.975	120.277	
B	1	1.034	62.734	0.154
	2	1.037	62.919	
	3	1.035	62.775	
C	1	0.516	30.995	0.608
	2	0.522	31.362	
	3	0.517	31.096	
D	1	0.225	13.201	0.087
	2	0.225	13.181	
	3	0.225	13.181	
E	1	0.106	5.911	1.308
	2	0.103	5.765	
	3	0.104	5.8	
F	1	0.052	2.649	1.249
	2	0.053	2.714	
	3	0.053	2.696	

Table 1. Acetaminophen concentrations of six unknown samples measured against a known standard curve. Percent coefficient of variation (%CV) was calculated using the concentration mean and standard deviation.

References

1) United States Pharmacopeia and Natural Formulary (USP 29 NF 24). Supplement No. 2. Rockville, MD: United States Pharmacopeia Convention; 2006: 3711

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