Quality Control of Small Molecule Pharmaceuticals Using Spectrophotometry

Key Words
NanoDrop 2000 Spectrophotometer, Acetaminophen, Pharmaceuticals, Purity, Quality Control

Introduction

The control of pharmaceutical product quality is essential to ensure safe and effective use in patients. The Thermo Scientific™ NanoDrop™ 2000 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 absorbance, and, in some instances, purity can also be monitored by analyzing spectral data.

In this study, UV absorbance spectra measured using a NanoDrop 2000 spectrophotometer were used to compare several acetaminophen samples from various sources with a known standard. In addition, reproducibility was assessed within each sample to determine the affects of aggregates or light scattering in the unknown samples.

The auto-ranging pathlength capabilities (Figure 1) of the NanoDrop 2000 spectrophotometer allow this instrument to quantify pharmaceutical samples across a much broader concentration range than is possible using a conventional cuvette-based spectrophotometer. By automatically selecting the optimum pathlength (ranging from 1.0 mm to 0.05 mm) the NanoDrop 2000 spectrophotometer can accurately measure the absorbance of a sample across a dynamic range nearly 300 fold greater than that of a cuvette based system.

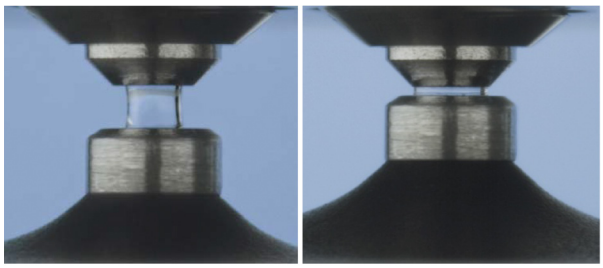

Figure 1: Sample liquid column during measurement, showing the 1.0 mm (left) and 0.1 mm (right) pathlengths.

Experimental Procedures

Test samples were analyzed per the US Pharmacopeia specifications that unknown concentrations of acetaminophen be quantified using absorbance at 244 nm against an acetaminophen standard curve prepared from a known standard of ≥99.9% purity. Prior to experimentation, the absorbance maximum of acetaminophen was confirmed as being at 244 nm (Figure 2).

Six samples of acetaminophen were compared to the standards. Each of these samples was dissolved in 100 mL deionized H_2O by incubating for 4 hours at room temperature on an orbital shaker (~100 rpm), resulting in ~5 mg/mL solutions.

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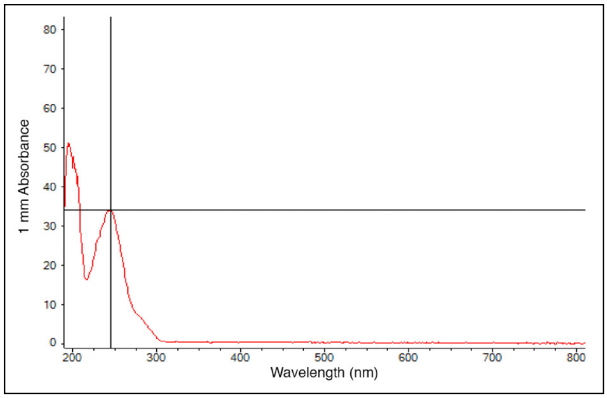

Figure 2: Absorbance spectrum of 6 mg/mL acetaminophen standard. Absorbance maximum at 244 nm was used for subsequent analysis.

A 6 mg/mL standard was prepared using 600 mg acetaminophen (Sigma-Aldrich, A7085) dissolved in 100 mL deionized H_2O. A standard curve was prepared by performing a serial dilution of this standard. Absorbance of triplicate, 1 µL aliquots of each dilution were measured at 244 nm using the NanoDrop 2000 UV-Vis application. A linear relationship between absorbance and concentration was observed throughout the entire standard curve range (Figure 3). Test acetaminophen solutions were measured against this standard curve in order to experimentally determine concentrations and calculate the total acetaminophen content.

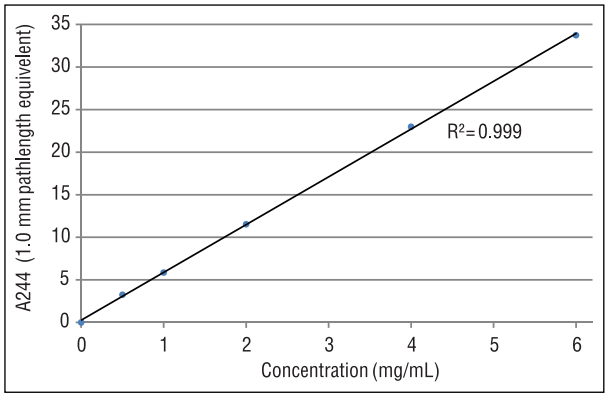

Figure 3: Standard curve of acetaminophen absorbance at 244 nm.

Results

Three separate solutions for each test acetaminophen sample were measured in triplicate to assess reproducibility (Table 1). Reproducibility was very good; CV values of less than 1% were observed in all cases.

Table 1: Acetaminophen content of six unknown samples measured against a known standard.

Sample	Replicate #	Average A244 (n=3)	Concentration (mg)	% CV
A	1	29.62	523.12	0.07
	2	28.02	494.67	0.34
	3	29.23	516.13	0.61
B	1	29.39	518.94	0.32
	2	29.26	516.73	0.35
	3	29.62	523.08	0.38
C	1	29.02	512.47	0.70
	2	28.61	505.13	0.34
	3	28.45	502.30	0.25
D	1	29.74	525.27	0.22
	2	29.63	523.20	0.17
	3	29.75	525.40	0.29
E	1	28.86	509.56	0.22
	2	29.78	525.93	0.83
	3	29.70	524.61	0.06
F	1	30.70	542.30	0.69
	2	30.36	536.24	0.38
	3	30.40	537.05	0.67

Absorbance spectra of the test acetaminophen samples (Figure 4) showed a nearly identical profile to the standard solution, shown in Figure 2.

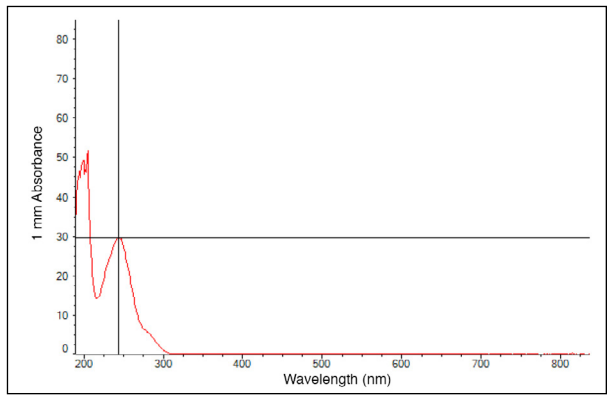

Figure 4: Absorbance spectrum of unknown acetaminophen sample "B". Nearly identical absorbance spectrum to that observed from measurements of the standard (Figure 2) suggests a pure acetaminophen sample.

Conclusion

The linear relationship between acetaminophen concentration and absorbance at 244 nm makes spectrophotometry an ideal method to verify batch concentrations. The Thermo Scientific NanoDrop 2000 spectrophotometer can be employed to provide a rapid and accurate spot-check of pharmaceutical products. The instrument's auto-ranging pathlength greatly reduces the need for sample dilution. The full spectrum display makes this instrument ideal for providing insight into the purity of various batches of product. Utilizing a traditional cuvette-based system, a greater number of large-volume serial dilutions would be required in order to dilute the samples from their stock concentrations to a measurable concentration range. By reducing the number of dilutions required, the NanoDrop 2000 spectrophotometer greatly reduces this potential source of error. In addition, the instrument's short measurement cycle and general ease of use greatly increases the rate at which samples can be processed, making it possible to implement multiple quality control checks throughout the production process.

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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