SHIMADZU

Application News

Spectrophotometric Analysis No.A411

Quantitation Limit of Pharmaceuticals Determined by UV-1800 UV-VIS Spectrophotometer

Quality control and securing product safety are important in the manufacture of pharmaceutical products, so it is essential that substances other than those specifically approved are not included with the specified ingredients. Cleaning validation is one of the measures specified in the GMP standard, because cleaning of manufacturing equipment is essential for preventing contamination and cross contamination of pharmaceutical products. This means that contaminants from the environment must not become mixed in with the product ingredients, and residual substances adhering to the manufacturing equipment must not contaminate the next product to be processed by that equipment. To verify these requirements, the cleaning itself must be evaluated, and the analytical instruments typically used for cleaning validation are the ultraviolet-visible spectrophotometer, the total organic carbon analyzer, and the high-performance liquid chromatograph.

The quantitation limit of an analytical instrument is the limit value at which residual sample can be quantitated. To determine whether or not the analytical instrument to be used for conducting cleaning validation can quantitate down to the permissible level of the residual substance, it is important to determine the quantitation limit. Here we introduce the determination of quantitation limit for the Shimadzu UV-1800 ultraviolet-visible spectrophotometer by absorption photometry, with samples consisting of detergent A used for cleaning in the pharmaceutical field, and the typically used pharmaceutical materials acetylsalicylic acid and isopropylantipyrine, presented along with the calculation method.

■ Determining the Quantitation Limit

One method of obtaining the quantitation limit is to determine the concentration value that corresponds to the absorbance which is 10 times the noise level1). The actual measurement method involves first measuring the absorption spectrum of a standard sample, and noting the wavelength of the greatest absorption peak. Next, measure the absorbance values at the wavelength of the greatest absorption peak using several samples of known concentration.

The slope of the calibration curve is determined from the relationship between the concentrations of the samples and the respective absorbance values. Lastly, repeat measurement of a blank sample (dilute solvent) is conducted and the standard deviation is obtained. The quantitation limit is calculated from the slope of the calibration curve and the value equivalent to 10 times the standard deviation. Determination of the quantitation limits of detergent A, acetylsalicylic acid and isopropylantipyrine according to this method are respectively presented below. The analytical conditions are as shown in Table 1.

Table 1 Analytical Conditions

Analytical instrument	: UV-1800
Measurement wavelength range	: 190 to 300 nm (detergent A) 250 to 350 nm (acetylsalicylic acid and isopropylantipyrine)
Scan speed	: Medium
Sampling pitch	: 1 nm
Photometric value	: Absorbance
Slit width	: 1 nm
Lamp switching wavelength	: 340 nm
Cell	: 10 mm quartz cell

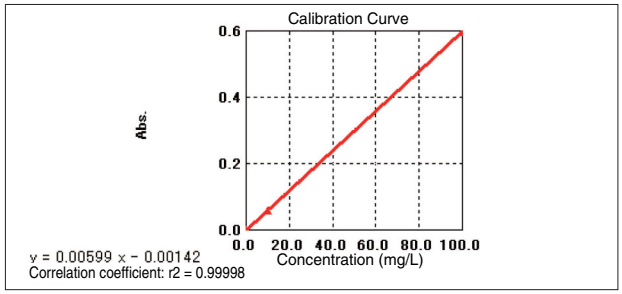

Fig.2 Calibration Curve of Detergent A

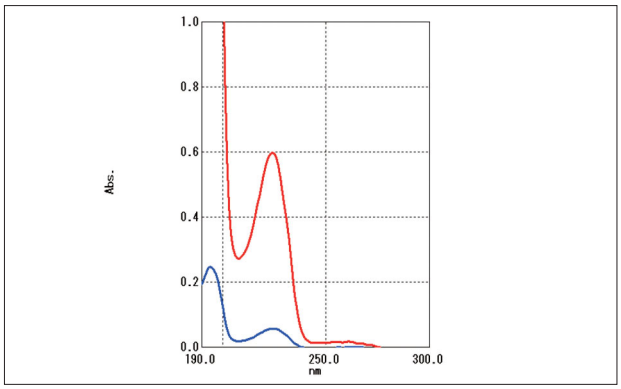

Fig.1 Absorption Spectra of Detergent A

Table 2 Absorbance of Blank Solution Measured Ten Times for Detergent A and Standard Deviation σ

Sample ID	WL225.0
1	0.00009
2	0.00020
3	0.00008
4	0.00011
5	0.00018
6	0.00012
7	0.00021
8	0.00034
9	0.00000
10	0.00006
Standard deviation σ	0.000096

■ Quantitation Limit of Acetylsalicylic Acid

Fig. 3 shows the absorption spectrum of acetylsalicylic acid methanol solution. The sample concentrations from higher-to-lower absorbance values are 400, 160, 80, 40, 20, and 8 mg/L. Fig. 4 shows the calibration curve at a measurement wavelength of 276 nm, and Table 3 shows the results of 10 repeat measurements of a blank sample and the standard deviation σ. The quantitation limit for acetylsalicylic acid is determined to be 0.42 mg/L.

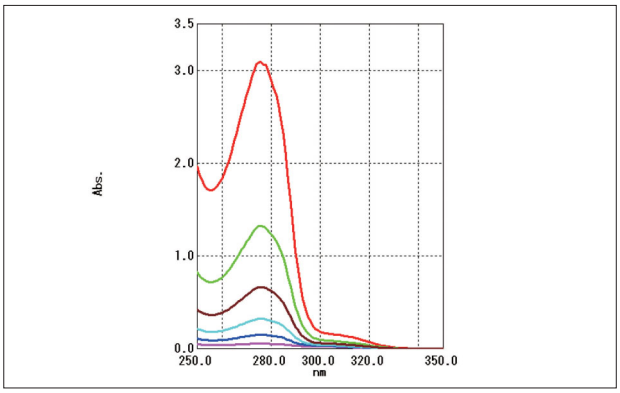

Fig.3 Absorption Spectra of Acetylsalicylic Acid Methanol Solution

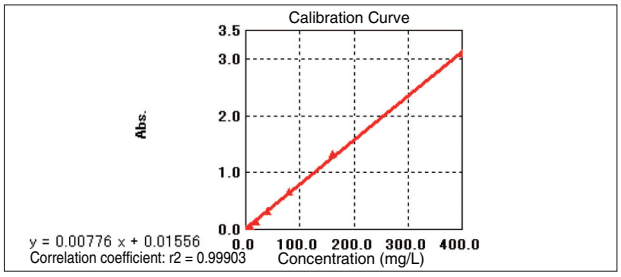

Fig.4 Calibration Curve of Acetylsalicylic Acid

Table 3 Absorbance of Blank Solution Measured Ten Times for Acetylsalicylic Acid and Standard Deviation σ

Sample ID	WL276.0
1	0.00009
2	-0.00018
3	-0.00024
4	0.00018
5	0.00067
6	0.00079
7	0.00037
8	0.00024
9	0.00021
10	0.00035
Standard deviation σ	0.000325

■ Quantitation Limit of Isopropylantipyrine

Fig. 5 shows the absorption spectrum of isopropylantipyrine methanol solution. The sample concentrations from higher-to-lower absorbance values are 80, 32, 16, 8, 4 and 1.6 mg/L. Fig. 6 shows the calibration curve at a measurement wavelength of 273 nm, and Table 4 shows the results of 10 repeat measurements of a blank sample and the standard deviation σ. The quantitation limit for isopropylantipyrine is determined to be 0.092 mg/L.

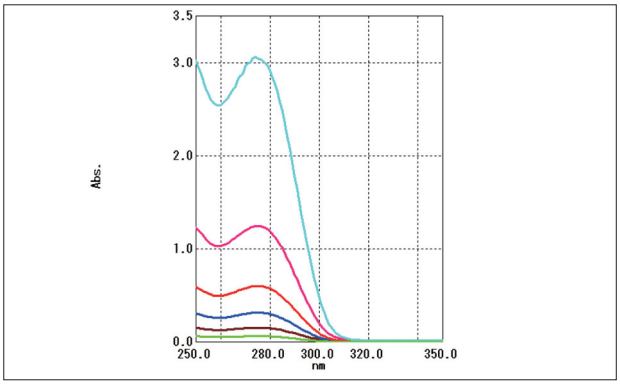

Fig.5 Absorption Spectra of Isopropylantipyrine Methanol Solution

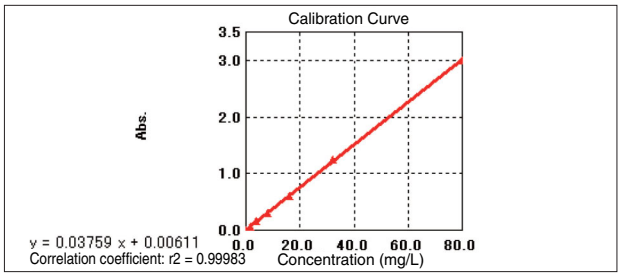

Fig.6 Calibration Curve of Isopropylantipyrine

Table 4 Absorbance of Blank Solution Measured Ten Times for Isopropylantipyrine and Standard Deviation σ

Sample ID	WL273.0
1	0.00000
2	0.00005
3	0.00032
4	-0.00024
5	-0.00020
6	0.00023
7	-0.00011
8	0.00095
9	0.00032
10	0.00006
Standard deviation σ	0.000347

■ Conclusions

The measurement results for detergent A, acetylsalicylic acid, and isopropylantipyrine were determined to illustrate the method of calculating quantitation limits based on measurements conducted using an UV-VIS spectrophotometer. Determination of the quantitation limit makes it possible to verify the lower limit of residual substances and residual detergent that can be quantitated. The validity of cleaning can be verified from various viewpoints using not just a UV-VIS spectrophotometer, but together with other types of instruments as well, including total organic carbon analyzers, high-performance liquid chromatographs, and high-performance liquid chromatograph mass spectrometers, for cleaning validation.

Reference Materials
1) Supervised by Shouji Hirai, "Fundamentals of On-Site Chemical Analysis, Chapter 7", edited by The Japan Society for Analytical Chemistry (2006), Ohmsha
- Yukitoshi Ikegami, "Cleaning Validation", PHARMA TECH JAPAN, Vol.17 No.2 (2001)
- Shimadzu Application News A403 "Measurement of Quantitation Limits of Vitamin B12 and Caffeine in Aqueous Solution Using UV-1800 UV-VIS Spectrophotometer"
- Shimadzu Application News O35 "Swab / Direct Combustion Analysis of Detergent Residue Using SSM-5000A (Cleaning Validation Application)"

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