SHIMADZU APPLICATION NEWS

LAAN-A-UV-E000A

● SPECTROPHOTOMETRIC ANALYSIS

No.A349A

Quantitative Analysis using Second-order Derivative Spectrum

Derivative spectral analysis is often used for the peak identification due to its advantages in differentiating closely adjacent absorption peaks, identifying weak absorption peaks obscured by sharp peaks, and identifying wavelength at maximum absorbance for broad spectra. Because the derivative values and concentration levels have a linear relationship, quantitation is easily performed even when background absorption exists.
This Application News presents an example of measurement using derivative spectrum with a UV-Vis spectrophotometer.

■ Derivative Spectrum

Derivative spectral analysis refers to taking the derivative with respect to absorbance wavelengths. Depending on the application, 1st, 2nd, 3rd or 4th-order derivative is conducted. In the past, derivative spectra were obtained using electronic methods (memory shift or analog differentiation) or optical methods (adjacent wavelength method or wavelength modulation method). However, currently, derivative spectra can be easily obtained by processing data on a Personal Computer.

The benefits of derivative spectral analysis include the following.

(1) Absorption bands can be identified even when two or more absorption peaks overlap in an extremely small wavelength range.
(2) Weak absorption bands can be identified even when obscured by a sharp absorbance peak.
(3) A particular wavelength at maximum absorbance can be identified from a broad absorption spectrum.
(4) There is a linear relationship between the derivative values and the concentration levels, so quantitative analysis is easily performed even when background absorption exists.

Fig. 1 shows a comparison of the original and second-order derivative spectra for two absorption peaks overlapping at extremely close wavelengths. (a) is the original spectrum, where absorption peak A overlaps with B to form the spectrum A + B. Here, it is impossible to identify absorption peak B. (b) is the second-order derivative spectrum. Sometimes the second-order derivative spectrum is flipped vertically to make it easier to see. In spectrum (b), absorption peak B, which was obscured by peak A, can be clearly identified. The peak-valley absorbance difference in the derivative spectrum relates linearly to concentration levels, making quantitation possible.

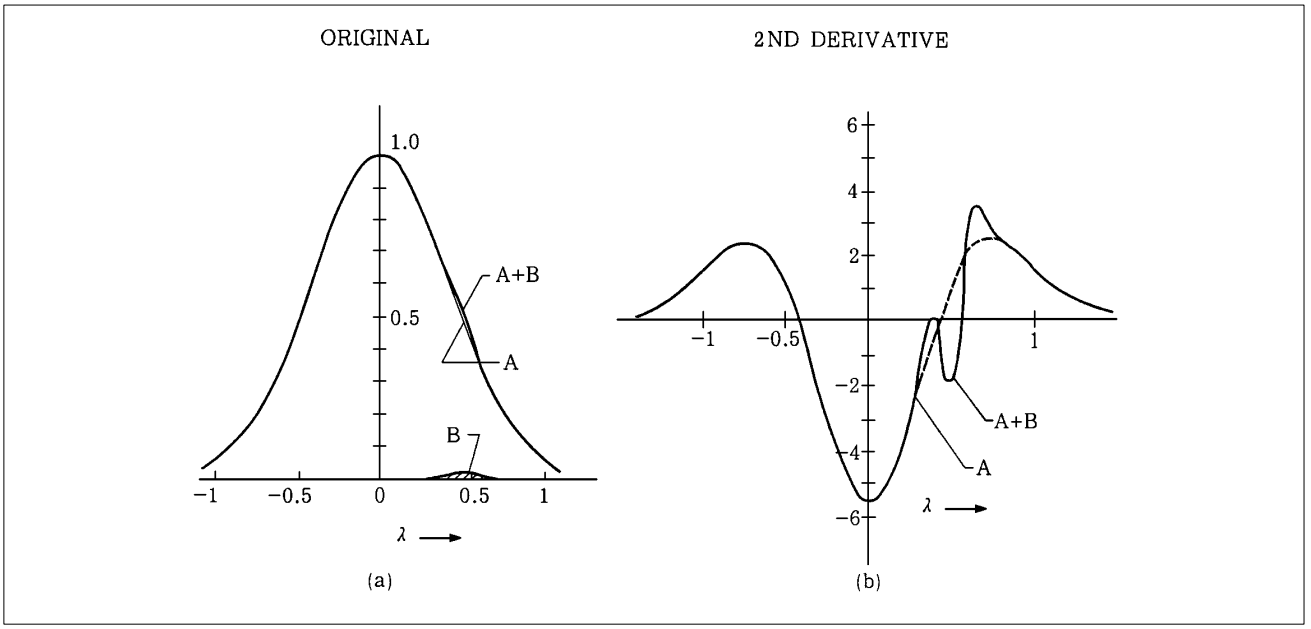

Fig.1 Original and 2nd-order Derivative Spectra

■ Quantitation of Paraquat and Diquat in Blood Plasma

If herbicides paraquat or diquat is ingested by mistake, they are absorbed by the body and can be detected in the blood plasma and urine. When measuring paraquat and diquat, their absorption peaks overlap in a small wavelength range. However, these two peaks can be differentiated by using the second-order derivative spectrum.[1)] A measurement of paraquat and diquat contained in blood plasma is shown below.

Fig. 2 shows the analysis flow chart. Fig. 3 and 4 show the measurement results. Excellent quantitation results were obtained for paraquat. Although not shown below, good results were also obtained for diquat. The measurement involved obtaining an absorption spectrum for the test sample and processing the data to calculate the second-order derivative spectrum. Based on the obtained second-order derivative spectrum, paraquat was quantified using the height between inflection points near 396nm and 403nm. Similarly, diquat was quantitated using the height between 454 nm and 464 nm.

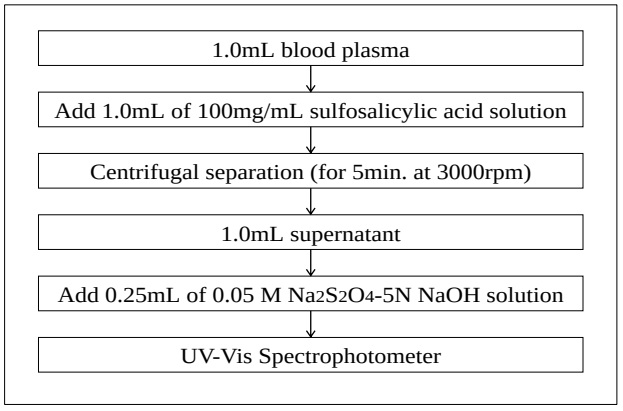

Fig.2 Flow Chart of Analytical Method for Paraquat and Diquat in Plasma

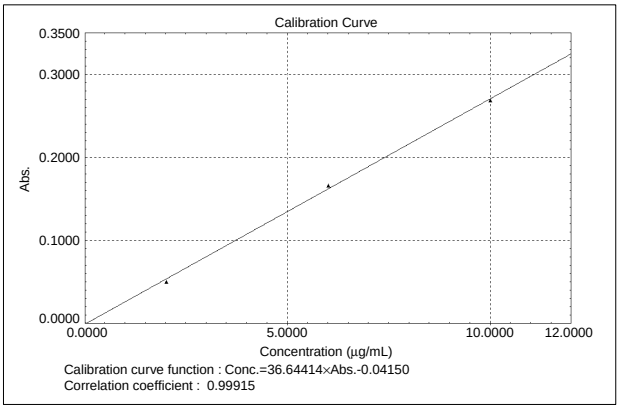

Fig.3 Determination of Paraquat in Plasma

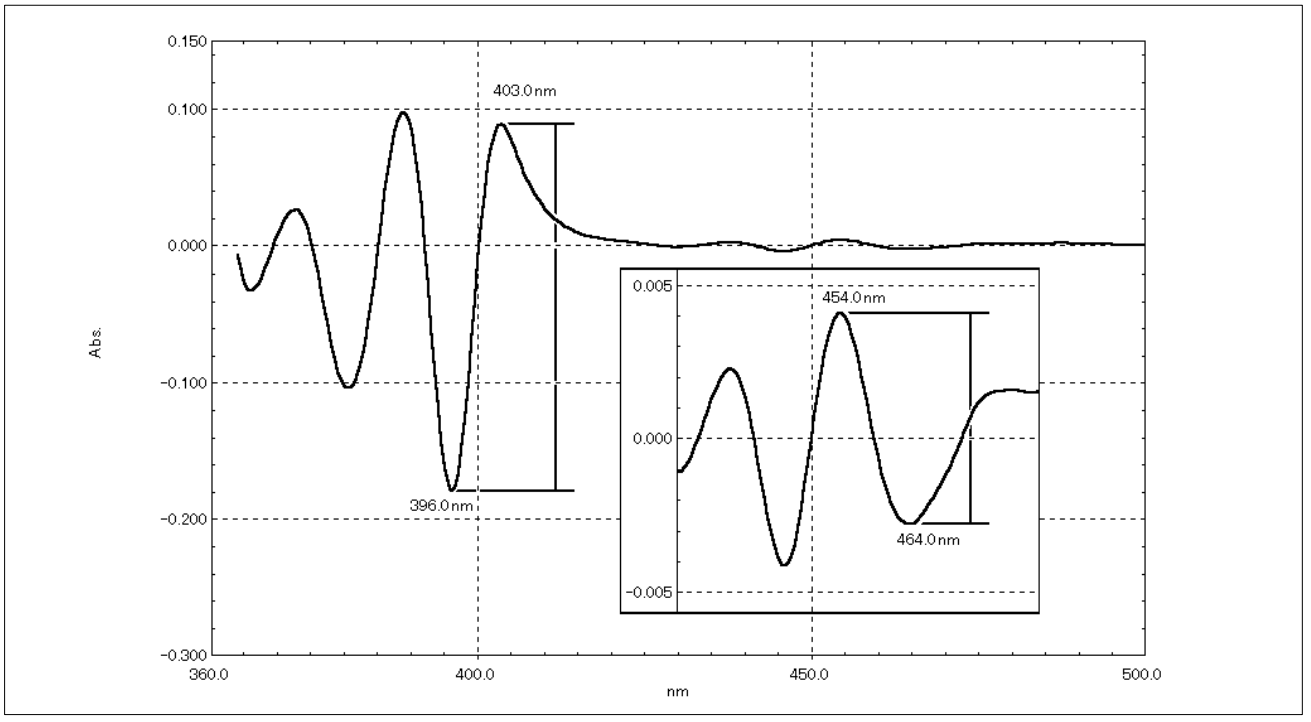

Fig.4 2nd Derivative Spectrum of Paraquat and Diquat in Plasma

Reference:
1) Chiaki Fukuie, Kiyoshi Ameno, Setsuko Ameno, et al.:
Simultaneous Analysis of Paraquat and Diquat Contained in Blood Serum and Urine using Second Derivative Spectrophotometry. Igaku No Ayumi, No. 7, Vol. 143, 1998, 657 - 658.

*The published data was not acquired using an instrument registered by Japanese pharmaceutical affairs law.

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-06401-10A-IK