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Application News

No. A578

Spectrophotometric Analysis

Ultraviolet-Visible Spectrophotometry Under JP/EP – Use of Evaluation Function of LabSolutions™ UV-Vis –

Ultraviolet-visible (UV-Vis) spectrophotometry is used in various fields and is also used in the validation tests stipulated in the various pharmacopoeias in the pharmaceutical field. In validation tests, the maximum absorption wavelength and the absorbances at a minimum of two wavelengths are compared. Thus, it is necessary to measure and analyze the spectra of samples.

The Spectra Evaluation Function of Shimadzu's recently-developed LabSolutions™ UV-Vis software enables automatic analysis and judgment after spectrometry and facilitates validation tests under various pharmacopoeias. This article introduces the Spectra Evaluation Function of LabSolutions™ UV-Vis and its application to pharmacopoeia validation tests.

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Spectra Evaluation Function

The Spectra Evaluation Function performs a predetermined analysis of the spectrometry results and displays a pass-fail judgment of the analysis results (evaluation value). Fig. 1 shows the Detail Settings window for the evaluation items, which includes 33 individual items under the 8 categories of Point Pick, Maximum Value, Minimum Value, Peak, Valley, Area, Statistics, and Cutoff.

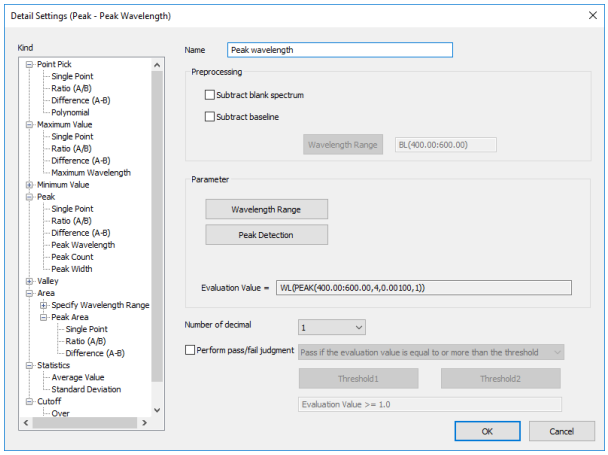

Fig. 1 Detail Settings Window for Evaluation Items

Validation Test Under Japanese Pharmacopoeia (JP)

The Japanese Pharmacopoeia (JP) stipulates the operations in connection with specific absorbance, purity tests, validation tests, and quantitation by UV-Vis spectrophotometry, depending on the sample. Here, a purity test of acetonitrile and validation tests of berberine chloride hydrate and rutin were performed under the conditions in Table 1.

Table 1 Measurement Conditions

Instrument	:	UV-1900
Wavelength range	:	200 to 250 nm (Acetonitrile) 200 to 400 nm (Berberine chloride hydrate) 220 to 400 nm (Rutin)
Scanning speed	:	Medium
Sampling pitch	:	1.0 nm

In the purity test of acetonitrile, pure water is measured as a comparison substance, and testing is performed to confirm values of 0.07 Abs max. at 200 nm, 0.046 Abs max. at 210 nm, 0.027 Abs max. at 220 nm, 0.014 Abs max. at 230 nm, and 0.009 Abs max. at 240 nm. As shown in Fig. 2, evaluation confirming the values at the respective wavelengths is possible by using "Single Point" under "Point Pick" in the Spectra Evaluation Function.

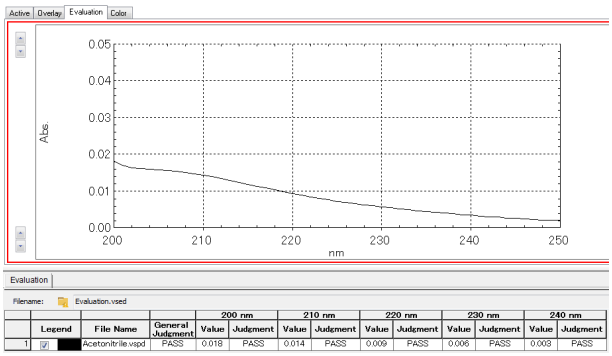

Fig. 2 Evaluation Results of Acetonitrile

In the validation test of berberine chloride hydrate, the maximum values of absorbance as a solution at 226 to 230 nm, 261 to 265 nm, and 342 to 346 nm are confirmed. As shown in Fig. 3, the peak wavelength in the wavelength range can be evaluated by using "Peak Wavelength" under "Peak" in the Spectra Evaluation Function.

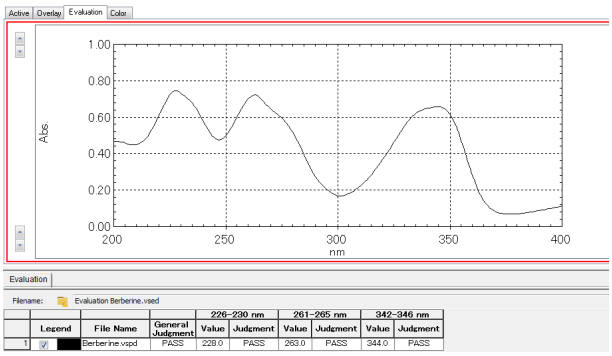

Fig. 3 Evaluation Results of Berberine Chloride Hydrate

In the validation test of rutin, the sample is dissolved in methanol, and the maximum values of absorbance at 255 to 259 nm and 356 to 360 nm are confirmed. Fig. 4 shows the results of the evaluation of rutin.

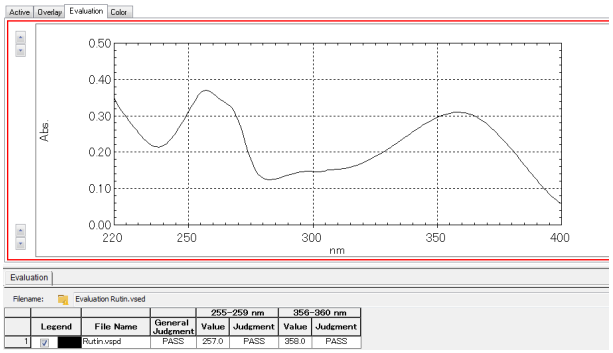

Fig. 4 Evaluation Results of Rutin

Validation Test Under European Pharmacopoeia (EP)

The European Pharmacopoeia (EP) stipulates the operation when using UV-Vis spectrophotometry, depending on the sample. IDENTIFICATION of rifampicin and TESTS of lactose monohydrate were performed under the conditions in Table 2.

In IDENTIFICATION of rifampicin, the sample is dissolved in methanol and then diluted with a phosphate buffer of pH 7.4, and a test is performed to confirm that the absorption maximum is shown at 237 nm, 254 nm, 334 nm, and 475 nm. In addition, A334/A475 of approximately 1.75 is also confirmed (A334/A475: ratio of values at wavelengths of 334 nm and 475 nm). As shown in Fig. 5, evaluation can be performed by verifying the peak wavelengths and absorbance ratio in the wavelength regions by using “Peak Wavelength” under “Peak” and “Ratio” under “Point Pick” of the Spectra Evaluation Function.

In TESTS of lactose monohydrate, the sample is dissolved in hot water, cooled, and diluted with pure water, and testing is performed to confirm that absorbance is 0.25 Abs max. at 210 to 220 nm, and is 0.07 Abs max. at 270 to 300 nm. Fig. 6 shows the results of the evaluation of lactose monohydrate.

Table 2 Measurement Conditions

Instrument	: UV-1900
Wavelength range	: 220 to 500 nm (Rifampicin) 210 to 300 nm (Lactose)
Scanning speed	: Medium
Sampling pitch	: 1.0 nm

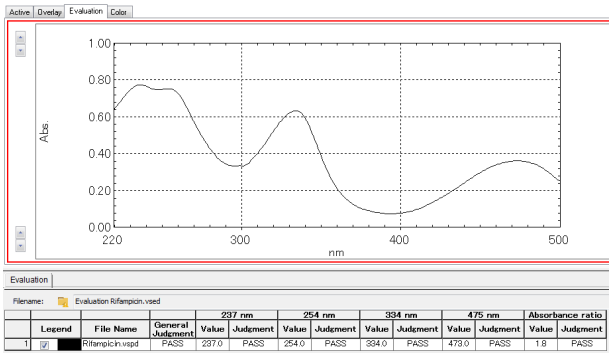

Fig. 5 Evaluation Results of Rifampicin

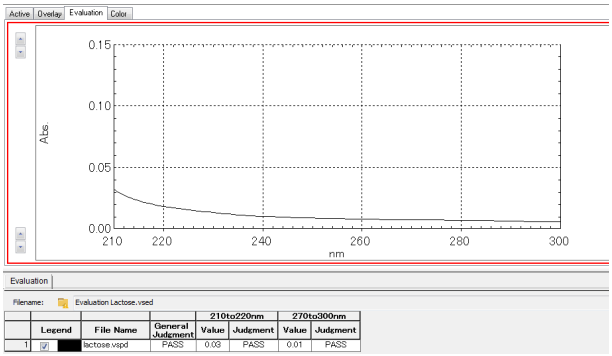

Fig. 6 Evaluation Results of Lactose Monohydrate

Conclusion

LabSolutions™ UV-Vis enabled simple testing and pass-fail judgments of various sample materials in tests described in JP and EP. The pharmacopoeias stipulate the performance requirements for instruments to be used in spectrometry. The Validation functions of UV-1900 can be used to confirm the required performance. For details concerning the Validation functions of UV-1900, refer to Application News No. A572.

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