

Application News

No. A594

Spectrophotometric Analysis

Identification of Pharmaceuticals Using the Spectral Evaluation Function of LabSolutions™ UV-Vis Software

: Japanese Pharmacopoeia Ultraviolet-Visible Spectrophotometry

Ultraviolet-visible spectrophotometers are used routinely in various industries for acceptance inspections of raw materials and quality inspections of products. In the pharmaceutical industry in particular, testing compliant with the Pharmacopoeia is prescribed as the criteria to determine whether or not the properties and quality of pharmaceuticals are appropriate. However, the testing requires burdensome tasks, such as reading specific peaks or calculating the absorbance ratio of multiple peaks from the obtained data, to judge the acceptability of the substances.

The LabSolutions™ UV-Vis software is equipped with the spectral evaluation function as a standard feature so that the tasks which generally require time and effort can be performed efficiently. This function automatically carries out analysis, including peak detection and calculation after spectrum measurement and makes a pass/fail judgment.

This article introduces the details of the spectral evaluation function using an example analysis for identification of "rutin, for thin-layer chromatography", which is described in the Japanese Pharmacopoeia (JP).

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■ Spectral Evaluation Function

The spectral evaluation function automatically carries out a pre-registered analysis on measurement results and enables pass/fail judgment to be made on these analysis results (evaluation values). Fig. 1 shows the detailed settings window of the spectral evaluation function.

Evaluation items include point pick, maximum value, minimum value, peak, valley, area, statistics, and cutoff, and the ability to use these items individually or in combination makes it possible to support all kinds of evaluations.

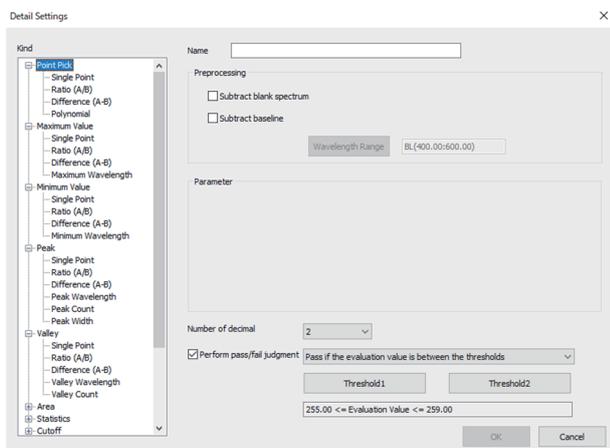


Fig. 1 Detailed Settings Window of the Spectral Evaluation Function

■ Identification of Rutin

Rutin is a vitamin-like substance which is expected to reinforce and strengthen capillary blood vessels in instances of high blood pressure. Fig. 2 shows the structural formula.

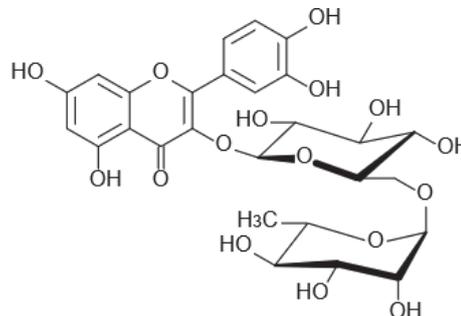


Fig. 2 Structural Formula of Rutin

The identification test of "rutin, for thin-layer chromatography" using ultraviolet-visible spectrophotometry is described in the JP as follows:

"Determine the absorption spectrum of a solution of rutin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry; it exhibits maxima between 255 nm and 259 nm, and between 356 nm and 360 nm."¹⁾

We performed the above identification test using the spectral evaluation function of LabSolutions UV-Vis software. The instrument used was a UV-1900 UV-VIS spectrophotometer. Fig. 3 shows the external appearance of the instrument and Table 1 lists the measurement conditions.



Fig. 3 UV-1900 UV-VIS Spectrophotometer

Table 1 Measurement Conditions

Measuring wavelength range	: 220 nm to 400 nm
Scan speed	: Medium speed
Sampling interval	: 1.0 nm
Slit width	: 1 nm
Light source changing wavelength	: 340 nm

First, the spectral evaluation items need to be set. This function allows setting of multiple items that can be evaluated at the same time. Fig. 4 shows the detailed settings window of the spectral evaluation function and Fig. 5 shows an enlarged view. In the identification test of "rutin, for thin-layer chromatography", a pass judgment is obtained when maxima between 255 nm and 259 nm and between 356 nm and 360 nm are exhibited. First, select [Peak] - [Peak Wavelength] ① under [Kind]. Next, set [Wavelength Range] ② under [Parameter] for the peak to be detected. In this example, the range was 220 to 400 nm. Select the [Perform pass/fail judgment] check box ③, set the judgment criteria, and select [Pass if the evaluation value is between the thresholds] ④ from the drop-down list. Lastly, set the [Threshold 1] and [Threshold 2] values ⑤, which define the acceptance range, as 255 nm and 259 nm respectively. Add an item for 356 nm to 360 nm using the same method.

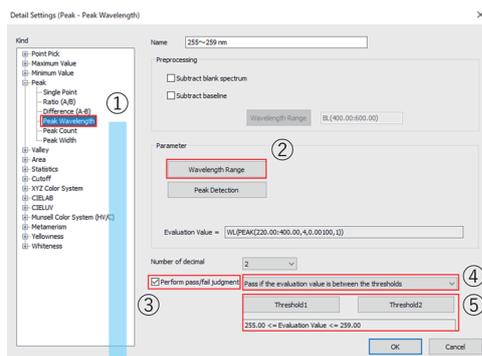


Fig. 4 Setting the Pass/Fail Judgment Criteria

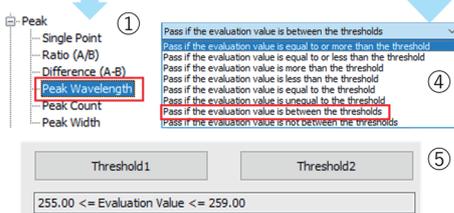


Fig. 5 Enlarged View of Evaluation Settings Window

① Evaluation Type ④ Evaluation Conditions ⑤ Threshold Settings

Click the [OK] button to set the spectral evaluation items shown in Fig. 6.

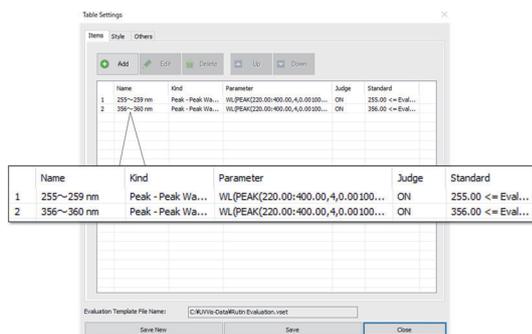


Fig. 6 List of Evaluation Items of "Rutin, for Thin-layer Chromatography"

References

- 1) Ministry of Health, Labour and Welfare of Japan: "Japanese Pharmacopoeia 17th Edition"
- 2) Shimadzu Corporation Application News No. A572

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Click the [Others] tab in Fig. 6 and select the [After measurement, data is automatically added to the evaluation table] check box to perform pass/fail judgment automatically after spectrum acquisition. Also, saving an evaluation template allows you to perform the same test at any time simply by loading the template. Since the JP specifies measurement of 1 g of "rutin, for thin-layer chromatography" diluted in 100,000 mL of methanol (1 in 100,000), a methanol solution prepared to 1.64×10^{-5} mol/L was measured. For comparison, a solution mixing trace wogonin as an impurity in the above solution, was also prepared and measured.

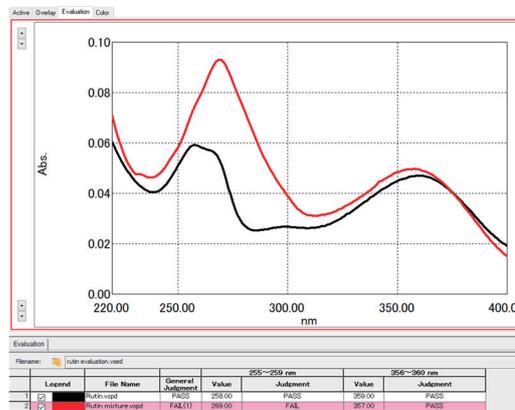


Fig. 7 Obtained Spectra and Evaluation Results

Legend		File Name	General Judgment	255~259 nm	356~360 nm		
1	2			Value	Judgment	Value	Judgment
1	2	Rutin.vsect	PASS	258.00	PASS	359.00	PASS
2	1	Rutin mixture.vsect	FAIL(1)	269.00	FAIL	357.00	PASS

Fig. 8 Enlarged View of Evaluation Results

Results

Fig. 7 shows the obtained spectrum and the evaluation results. (Fig. 8 shows an enlarged view of the evaluation results.) The black line is the methanol solution of "rutin, for thin-layer chromatography" and the red line is the mixed solution containing the impurity. The "rutin, for thin-layer chromatography" solution received a "PASS" for both evaluation items. On the other hand, the mixed solution received a "FAIL" and is colored red in the table because a peak at 258 nm is absent due to the impurity. The column color in the evaluation result table changes when a result deviates from the set evaluation criteria and this allows you to determine at a glance whether a test passed or failed. Moreover, this table information can be copied into other applications using the copy and paste function or the [Excel Export] function to facilitate creation of various reports.

Conclusion

This article introduced an example analysis to demonstrate how the identification testing compliant with the JP can be performed efficiently by using the spectral evaluation function of the LabSolutions UV-Vis software. The use of this function enables identification testing conforming to various standards with predetermined criteria. The Pharmacopoeia of each country also describes the performance of instruments to be used for spectrophotometry. Shimadzu provides validation functions compliant with JP, EP (European Pharmacopoeia), and USP (United States Pharmacopoeia). For details on the UV-1900, refer to Application News No. A572²⁾.



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