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Application News

UV-Visible Spectrophotometer, Spectrofluorophotometer, Fourier Transform Infrared Spectrophotometer, and Infrared Raman Microscope

Multifaceted Spectrophotometric Analysis of Vitamins and Vitamin-like Substances

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User Benefits

- Ultraviolet-visible spectrophotometers can measure the light absorption properties, ranging from ultraviolet to visible light, of liquids, and spectrofluorophotometers can measure the fluorescent light properties of liquids and powders.
- Fourier transform infrared spectrophotometers and Raman spectrophotometers enable easy qualitative analysis of substances that are in liquid or powdered states.
- Spectrophotometric properties can be investigated easily by using a variety of spectrophotometric methods, depending on the sample.

■ Introduction

Vitamins help to maintain proper bodily functions, but almost none of them can be synthesized within the body, so they must be obtained from foods. Generally, vitamins can be classified as either water-soluble vitamins, which dissolve easily in water, or fat-soluble vitamins, which do not. Vitamin-like substances are nutrients that serve a similar function to vitamins and can be synthesized within the body. Both vitamins and vitamin-like substances, which are essential to humans, require analytical instruments to determine which substances contain them and to what degree. Information about simultaneous analysis of water-soluble and fat-soluble vitamins is included in Analytical Solutions for Food Development.[1)] This article provides an overview of measuring vitamins and vitamin-like substances using the spectrophotometric instruments shown in Fig. 1 (a UV-VIS spectrophotometer, a spectrofluorophotometer, a Fourier transform infrared spectrophotometer, and a Raman spectrophotometer), and it describes some examples.

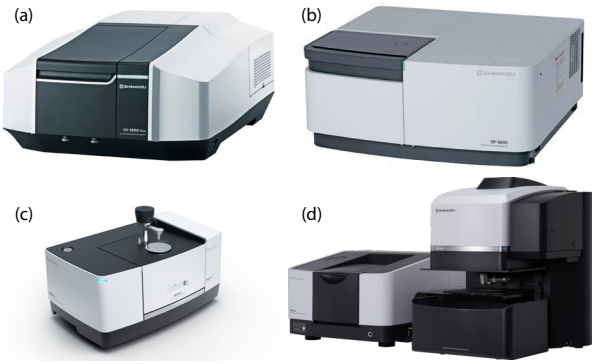

Fig. 1 (a) UV-2600i Plus UV-Visible Spectrophotometer
(b) RF-6000 Spectrofluorophotometer
(c) IRSpirit™-X Fourier Transform Infrared Spectrophotometer
(d) IRXross™ Fourier Transform Infrared Spectrophotometer
+ AIRsight™ Infrared Raman Microscope

■ Samples and Measurement Conditions

To enable measurements with the UV-Visible spectrophotometer and spectrofluorophotometer, samples were prepared by dissolving each type of reagent (vitamins and vitamin-like substances) in a solvent. For the UV-visible spectrophotometer, concentrations were adjusted to ensure 1 Abs or less absorbance, and for the spectrofluorophotometer, they were diluted, as necessary. The respective measurement conditions are listed in Tables 1 and 2.

The Fourier transform infrared spectrophotometer (FTIR) and Raman microscope can measure powder and solid samples directly without pretreatment. (Samples are pressed against a prism for the ATR method.) The quantities of the liquid samples were adjusted to prevent evaporation before measuring. The respective measurement conditions are listed in Tables 3 and 4.

Table 1 UV-Vis Spectrophotometer Measurement Conditions

Instrument:	UV-2600i Plus
Measurement Wavelength Range:	200 – 800 nm (solvent: purified water) 220 – 800 nm (solvent: ethanol) 230 – 800 nm (solvent: 0.1 M NaOH) 240 – 800 nm (solvent: chloroform)
Data Interval:	1.0 nm
Scan Speed:	Medium
Slit Width:	1.0 nm

Table 2 UV-Visible Spectrophotometer Measurement Conditions

Instrument:	RF-6000
Excitation (Ex) Wavelength:	UV-visible spectrophotometer peak wavelengths (refer to Table 5)
Fluorescence (Em) Wavelength Range:	Graph range
Data Interval:	Em 1.0 nm
Scan Speed:	200 nm/min
Spectral Bandwidth:	Ex 5.0 nm, Em 5.0 nm
Sensitivity:	Low

Table 3 FTIR Measurement Conditions

Instruments:	IRSpirit-TX and QATR-S (diamond)
Resolution:	4 cm^{-1}
Number of scans:	40
Apodization function:	Happ-Genzel
Detector:	DLATGS

Table 4 Raman Measurement Conditions

Instruments:	IRTracer-100, AIRsight
Raman Spectrophotometry Measurements	
Number of scans:	10
Exposure time:	5.0 sec
Objective lens:	x100 magnification
Excitation wavelengths:	532 nm and 785 nm
Detector:	CCD

■ Measurement Results

The spectrophotometric measurements for vitamins and vitamin-like substances are shown in Figs. 2 to 21. The UV-visible spectrophotometric data have been normalized based on major peaks. Infrared and Raman spectra were adjusted to show peak positions using auto-scaling based on their respective intensity levels.

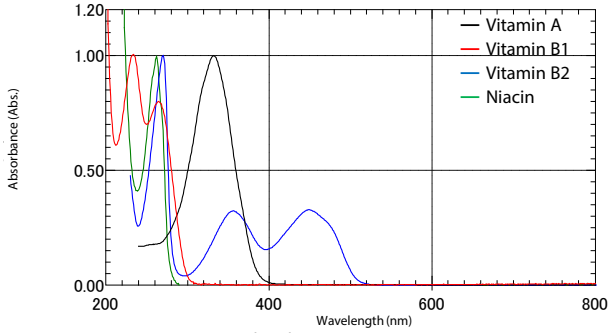

Fig. 2 Absorbance Spectra 1

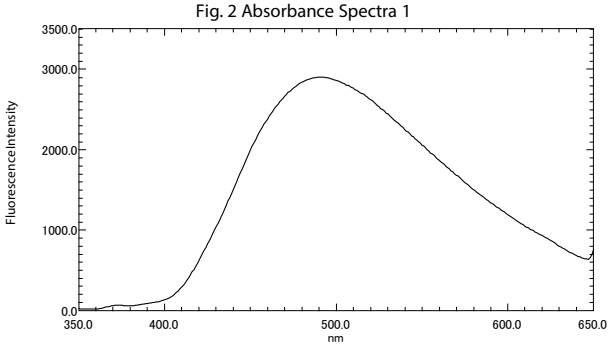

Fig. 3 Example of Fluorescence Spectrum for Vitamin A (Ex332 nm)

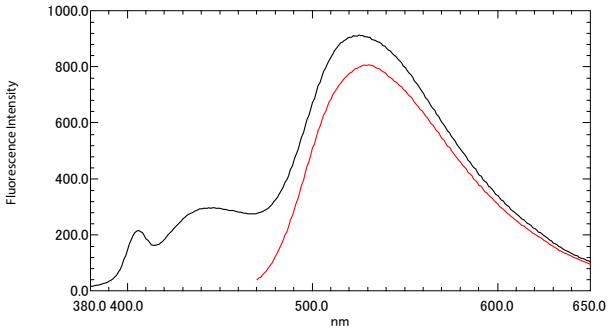

Fig. 4 Fluorescence Spectra for Vitamin B2 – Black: Ex356 nm, Red: Ex449 nm

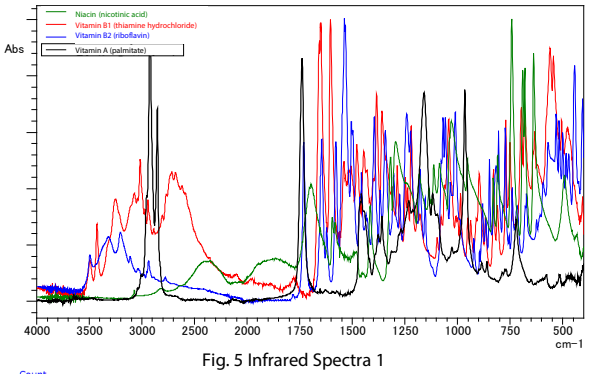

Fig. 5 Infrared Spectra 1

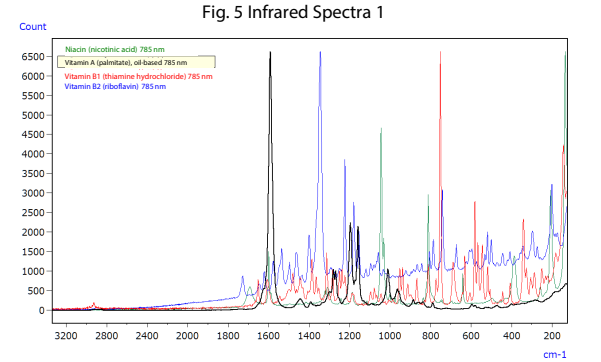

Fig. 6 Raman Spectra 1 (Ex785 nm)

Vitamin B2 appeared orange in powder form and had absorption peaks outside the UV range. Vitamins A and B2 exhibited fluorescent light. However, the fluorescence intensity of vitamin A near 490 nm was unstable, with the levels fluctuating. That may have been a reaction to the excitation light. The fluorescent light confirmed at 440 nm, when vitamin B2 was excited with 356 nm light, was also similarly unstable.

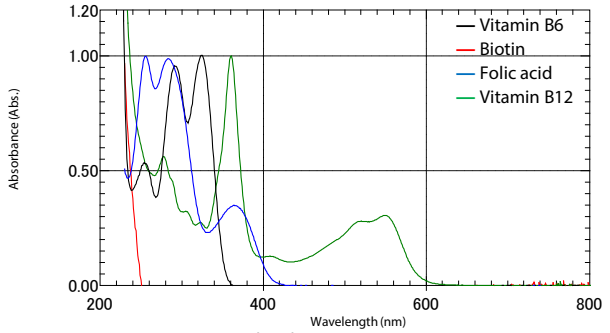

Fig. 7 Absorbance Spectra 2

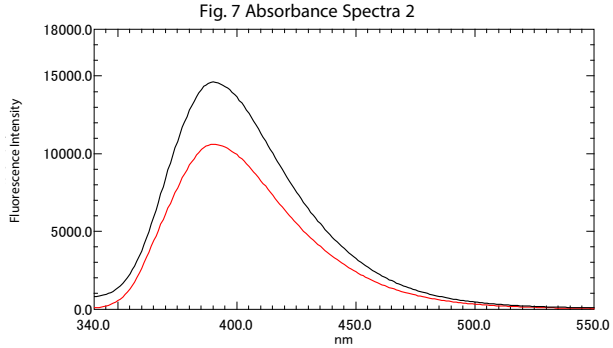

Fig. 8 Fluorescence Spectra for Vitamin B6 – Black: Ex291 nm, Red: Ex324 nm

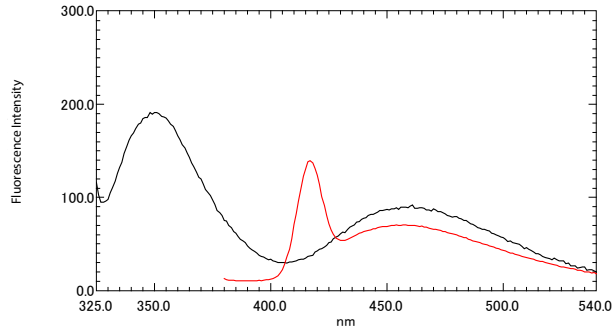

Fig. 9 Fluorescence Spectra for Folic Acid – Black: Ex284 nm, Red: Ex365 nm

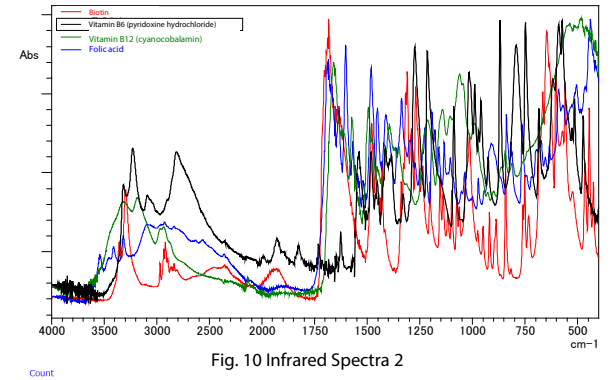

Fig. 10 Infrared Spectra 2

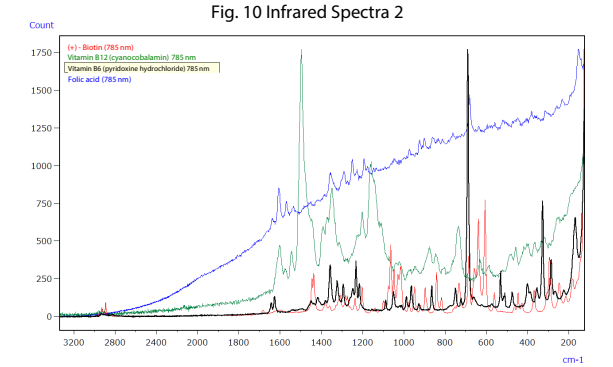

Fig. 11 Raman Spectra 2 (Ex785 nm)

Vitamin B12 appeared red in powder form and had absorption peaks outside the UV range. Vitamins B6 and folic acid exhibited fluorescent light, confirming that different fluorescence spectra appeared depending on the excitation wavelength. Also, due to the strong fluorescent light intensity of the powdered form, the baseline increased even in the Raman spectra shown in Fig. 11.

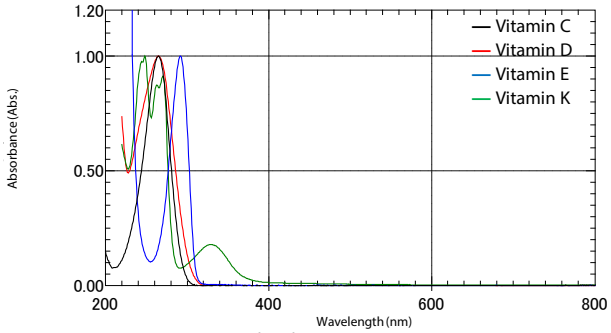

Fig. 12 Absorbance Spectra 3

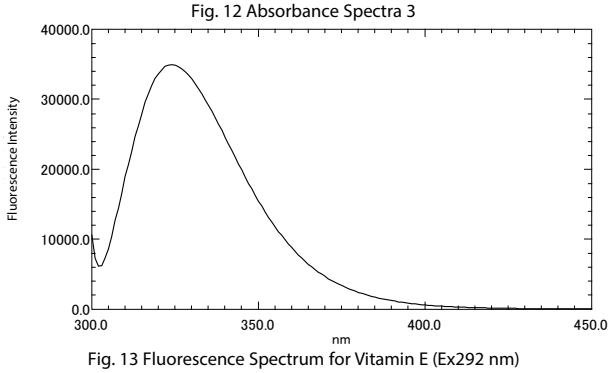

Fig. 13 Fluorescence Spectrum for Vitamin E (Ex292 nm)

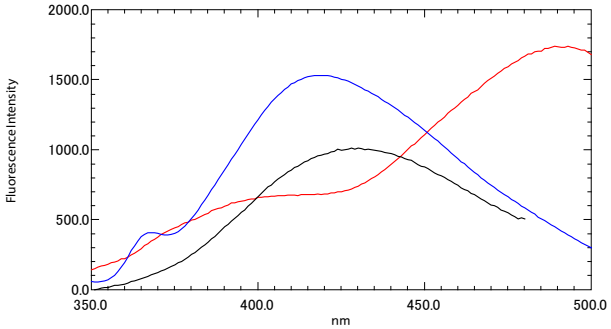

Fig. 14 Fluorescence Spectra for Vitamin K
– Black: Ex248 nm, Red: Ex270 nm, and Blue: Ex330 nm

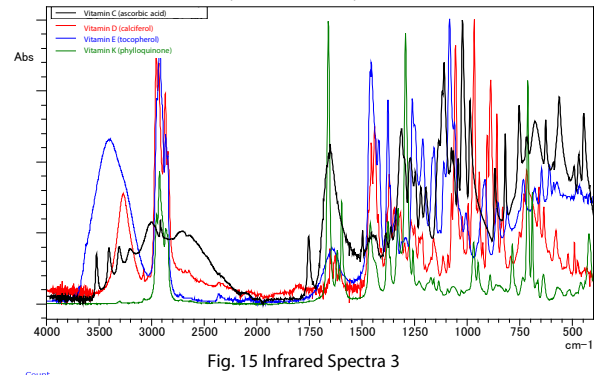

Fig. 15 Infrared Spectra 3

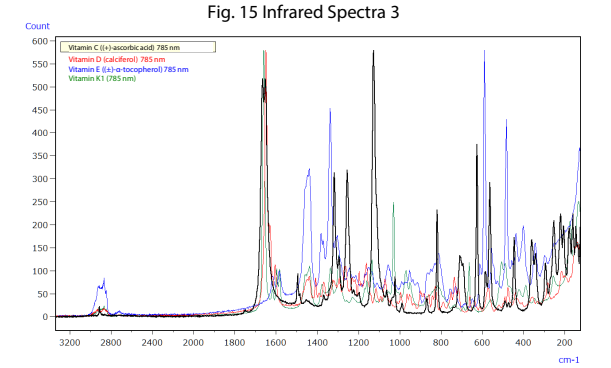

Fig. 16 Raman Spectra 3 (Ex785 nm)

Vitamins E and K exhibited fluorescent light. The vitamin K signal was unstable, particularly at the excitation wavelength of 270 nm. In addition to the fluctuations in fluorescence intensity, there were also variations in the presence or absence of peaks. Consequently, the data in Fig. 14 should only be used for reference purposes.

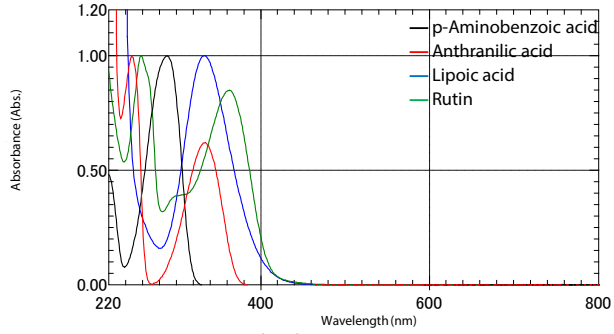

Fig. 17 Absorbance Spectra 4

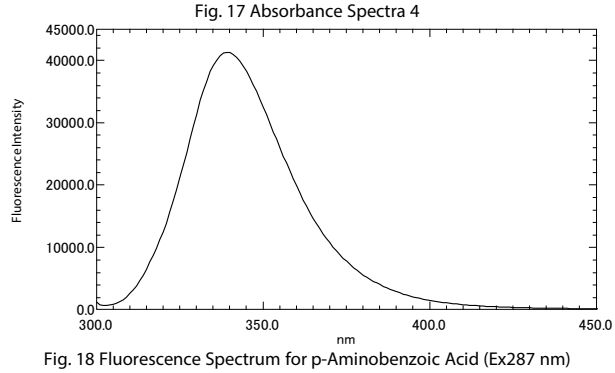

Fig. 18 Fluorescence Spectrum for p-Aminobenzoic Acid (Ex287 nm)

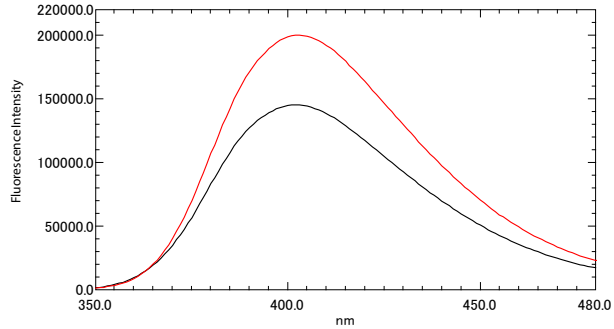

Fig. 19 Fluorescence Spectra for Anthranilic Acid
– Black: Ex333 nm and Red: Ex248 nm

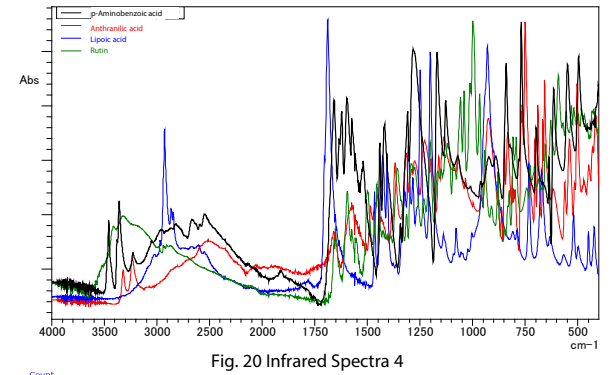

Fig. 20 Infrared Spectra 4

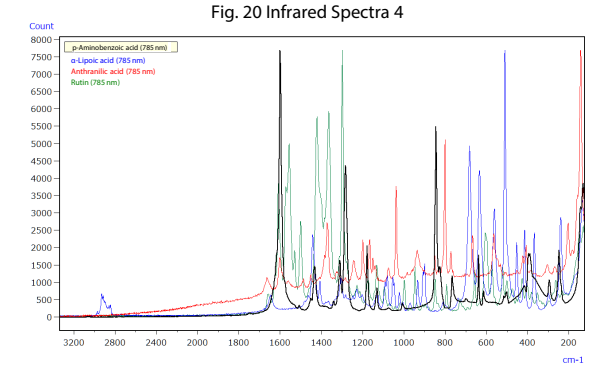

Fig. 21 Raman Spectra 4 (Ex785 nm)

Among the vitamin-like substances, p-aminobenzoic acid and anthranilic acid exhibited fluorescent light. Anthranilic acid exhibited stronger fluorescence intensity for the excitation wavelength of 333 nm.

Table 5 Optical Properties of Vitamins and Vitamin-like Substances

Name	Reagent Measured	Solvent	Absorption in UV Region	Presence of Fluorescent Light*1	Infrared Spectrum	Raman Spectrum*4 Excitation Wavelengths: 532 nm and 785 nm
Vitamin A	Vitamin A (palmitate)	Chloroform	332 nm	About 490 nm*2	○*3	○/○
Vitamin B1	Thiamine hydrochloride	Purified water	234/265 nm	△	○*3	△/○
Vitamin B2	Riboflavin	Sodium hydroxide	270/356/449 nm	About 440*2/530 nm	○	×/○
Niacin	Nicotinic acid	Purified water	262 nm	△	○	△/○
Vitamin B6	Pyridoxine hydrochloride	Purified water	291/324 nm	390 nm	○	△/○
Biotin	Biotin	Sodium hydroxide	–	–	○	○/○
Folic acid	Folic acid	Sodium hydroxide	256/284/364 nm	350/458 nm	○	×/△
Vitamin B12	Cyanocobalamin	Purified water	278/361 nm	–	○	×/○
Vitamin C	Ascorbic acid	Purified water	265 nm	–	○	○/○
Vitamin D	Calciferol	Ethanol	265 nm	–	○	○/○
Vitamin E	α-Tocopherol	Ethanol	292 nm	324 nm	○*3	○/○
Vitamin K	Phylloquinone	Ethanol	248/270/330 nm	Yes (unstable)	○*3	×/○
Vitamin-like substance	p-Aminobenzoic acid	Ethanol	289 nm	339 nm	○	○/○
Vitamin-like substance	Anthranilic acid	Ethanol	247/334 nm	403 nm	○	△/○
Vitamin-like substance	Lipoic acid	Ethanol	333 nm	–	○*3	○/○
Vitamin-like substance	Rutin	Ethanol	258/363 nm	–	○	△/○

*1: The △ symbol in the "Presence of Fluorescent Light" column indicates a weak signal.
*2: Due to easily bleaching, fluorescence wavelengths are marked "about" and indicate approximate regions.
*3: Spectra that are not in the Shimadzu library
*4: The △ symbol in the "Raman Spectrum" column indicates spectra affected by fluorescent light.
The × symbol indicates Raman spectra that are not in the library because they are difficult to observe.

Table 5 indicates the reagents measured, the solvents used, the absorption peak wavelengths in the UV region, the presence of fluorescent light, and the presence of infrared/Raman spectra.

■ Conclusion

Vitamins and vitamin-like substances were measured using a UV-visible spectrophotometer, a spectrofluorophotometer, a Fourier transform infrared spectrophotometer, and a Raman microscope (a Raman spectrophotometer). UV-visible spectroscopy measures absorption wavelengths in the UV region for dissolved samples and can also be used for quantitative measurements. The spectrofluorophotometer can determine the presence of fluorescent light to identify optical properties, or, depending on the sample, it can measure trace quantities.[2] The FTIR and Raman results showed that library data can be easily used for identification analysis.

Shimadzu offers a wide range of spectrophotometric devices that can investigate the various spectroscopic properties of substances.

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2. Difference in Quantifiable Concentration Ranges of UV-Vis Spectrophotometer and Fluorescence Spectrophotometer Application News No. A615

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