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UV-VIS Spectrophotometer

Application News

Chlorophyll Quantitative Measurement: Using Equations from Japanese Water Standard Test Methods

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User Benefits

- In quantitation/photometric mode, LabSolutions™ UV-Vis can now be configured with coefficients on a per-sample basis when calculating concentration.
- Quantitation/photometric mode has been updated to support a wider range of mathematical functions (power, square root, common logarithm, and natural logarithm).

■ Introduction

The tap water we use every day is subject to water quality controls that include Japanese water standard test methods. These water standard test methods are mainly performed on GC-MS, LC-MS, and HPLC systems, although absorption spectrophotometry using a spectrophotometer is used to test for some substances. The quantification of concentration is typically determined based on calibration curves, although some substance concentration is determined based on complex equations. These complex equations can sometimes require the user to manually transfer measurement data into an Excel sheet. The risk of human error associated with this manual transfer can be avoided if the software being used to control the analysis instrument can also be configured to perform these complex equations.

The updated LabSolutions™ UV-Vis Ver. 1.21 now offers an even greater set of features for configuring highly complex equations. This article describes using equations in LabSolutions UV-Vis to calculate the concentration of chlorophyll as outlined in Japanese Water Standard Test Methods III: Organic Materials.

Fig. 1 UV-1900i Plus Spectrophotometer

■ Sample Preparation and Absorption Spectrophotometry

The acetone extraction and ethanol extraction methods described in Japanese Water Standard Test Methods were used to analyze municipal water from a building, water from a drinking fountain in a park, and liquid extracted from a cabbage leaf.*1

Acetone extraction was performed by adding 1 mL of magnesium carbonate suspension to 1 liter of test water*2, mixing, then filtering the mixture under suction through glass fiber filter paper. The residue captured by the filter paper was cut into small pieces, placed in a mortar, pulverized together with 2 to 3 mL of acetone, and the mixture transferred to a 15 mL centrifuge tube. The mortar was also cleaned several times with 2 to 3 mL of acetone and added to the same centrifuge tube, making up 12 mL of acetone in total. After leaving the centrifuge tube to stand for 1 hour in a cool, dark place, the mixture was centrifuged at around 1500 g for 10 minutes. The supernatant was placed in a separate container, where 10 mL of the supernatant was used as the test solution.

*1 Not subject to testing under Japanese Water Standard Test Methods, but included as a reference. About 0.1 g or 2 cm^2 of an outside cabbage leaf was used. The extraction method is described in more detail in Application News No. A631.[1)]

*2 Japanese Water Standard Test Methods also specify appropriate analyte levels, which are between 2 and 20 µg for chlorophyll a.

Ethanol extraction was performed by shaking 1 liter of test water*2 and filtering under suction through glass fiber filter paper. The residue captured by the filter paper was then cut into small pieces. A fixed volume of ethanol (20 or 25 mL) was also placed in an extraction container, then the filter paper was soaked in the ethanol, shaken a little, and suspended again. The extraction container was then immersed in a water bath (75 °C ± 1 °C) and heated for 5 minutes. The extraction container was removed from the water bath and allowed to cool to room temperature for around 15 minutes. The contents of the extraction container were centrifuged for long enough to produce a supernatant, and the supernatant was used as the sample solution.

■ Calculating Concentration by Acetone Extraction

The UV-1900i Plus spectrophotometer shown in Fig. 1 was used to analyze samples under the conditions shown in Table 1. Acetone was also used as a control (blank).

Table 1 Measurement Conditions

Equipment:	UV-1900i Plus
Slit Width:	1 nm
Photometric Data:	Absorbance
Photometric Mode	
Measured Wavelengths:	480, 630, 645, 663, and 750 nm
Integration Time:	1.0 sec
Spectral Mode	
Measured Wavelength Range:	400 to 760 nm
Scanning Speed:	Low

Subtracting the absorbance at 750 nm from the absorbance at 630, 645, and 663 nm gives e_{630}, e_{645}, and e_{663}, respectively, for a 10-mm absorption cell. Water Standard Test Methods defines the concentration of chlorophyll a using equation (1) below. The volume of test solution and test water must be entered into this equation for each sample analysis.

$$\text{Chlorophyll a (µg/L)} = (11.64e_{663} - 2.16e_{645} + 0.10e_{630}) \times \frac{\text{Test solution } (mL)}{\text{Test water } (mL)} \times 1000 \quad (1)$$

The concentration of plant carotenoids can also be determined using equation (2), where e_{480} is the absorbance at 750 nm subtracted from the absorbance at 480 nm.

$$\text{Plant carotenoids} = 4.0e_{480}$$
when the sample contains large amounts of green algae or blue–green algae (2)
$$\text{Plant carotenoids} = 10.0e_{480}$$
when the sample contains large amounts of diatoms

Using acetone extraction, the concentration of chlorophyll b and c can also be calculated using equations (3) and (4) below.

$$\text{Chlorophyll b (µg/L)} = (-3.94e_{663} + 20.97e_{645} - 3.66e_{630}) \quad (3)$$

$$\text{Chlorophyll c (µg/L)} = (-5.53e_{663} - 14.81e_{645} + 54.22e_{630}) \quad (4)$$

Fig. 2 shows a LabSolutions UV-Vis window configured with equations (1) through (4). Starting with LabSolutions UV-Vis Ver. 1.21, the factor settings window can now be used to add columns for entering the volume of test solution and test water. Fig. 3 shows data measured in photometric mode and the calculated results, and Fig. 4 shows example spectra recorded in spectral mode.

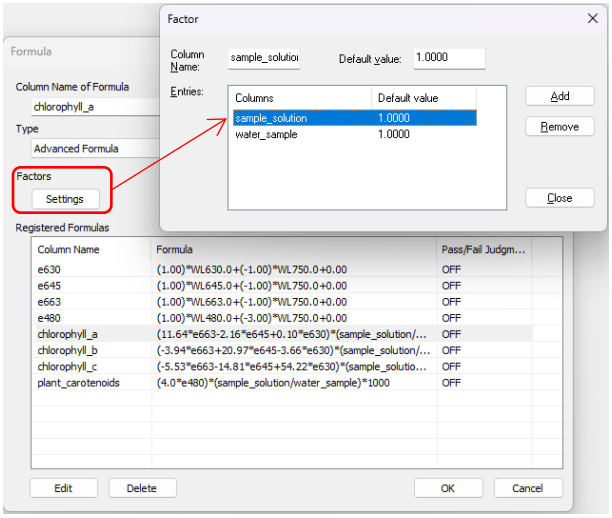

Fig. 2 LabSolutions UV-Vis Formula Window: Acetone Extraction Equations

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	Sample Name	WL630.0	WL645.0	WL663.0	WL480.0	WL750.0	e630	e645	e663	e480	chlorophyll_a	chlorophyll_b	chlorophyll_c	plant_carotenoids	sample_solution	water_sample
1	tap water1	0.0007	0.0007	0.0007	0.0007	0.0020	−0.0013	−0.0013	−0.0013	−0.0053	−0.1245	−0.1738	−0.4404	−0.2120	10.0000	1000.0000
2	tap water2	0.0014	0.0013	0.0013	0.0022	0.0023	−0.0009	−0.0010	−0.0010	−0.0047	−0.0957	−0.1374	−0.2846	−0.1880	10.0000	1000.0000
3	drinking fountain1	0.0010	0.0010	0.0009	0.0016	0.0020	−0.0010	−0.0010	−0.0011	−0.0044	−0.1074	−0.1298	−0.3333	−0.1760	10.0000	1000.0000
4	drinking fountain2	0.0009	0.0009	0.0009	0.0014	0.0020	−0.0011	−0.0011	−0.0011	−0.0046	−0.1054	−0.1471	−0.3727	−0.1840	10.0000	1000.0000
5	extracted cabbage leaves1	0.0016	0.0031	0.0095	0.0051	0.0005	0.0011	0.0026	0.0090	0.0036	49.6270	7.5180	−14.3170	7.2000	10.0000	20.0000
6	extracted cabbage leaves2	0.0011	0.0016	0.0043	0.0035	0.0011	0.0000	0.0005	0.0032	0.0002	18.0840	−1.0615	−12.5505	0.4000	10.0000	20.0000

Fig. 3 Photometric Results Window: Acetone Extraction

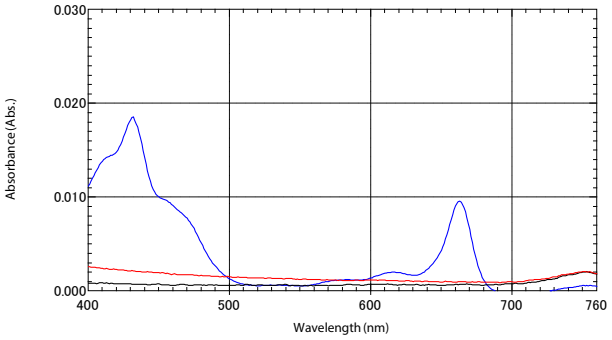

Fig. 4 Acetone Extraction: Example Chlorophyll Spectra
Black line: municipal water, red line: park water,
blue line: cabbage leaf extract

No chlorophyll a was detected in the municipal water from the building or in the water from a park drinking fountain. A peak was recorded at 663 nm in the cabbage leaf spectrum (Fig. 4) and used to calculate the concentration of chlorophyll a. As shown in Fig. 3, the volume of test solution and test water can be entered on a row-by-row basis, and LabSolutions UV-Vis successfully handled the complex equations shown in equations (1) through (4).

■ Calculating Concentration by Ethanol Extraction

Table 2 shows the conditions used for analysis. Ethanol was used as a control (blank).

Table 2 Measurement Conditions

Equipment:	UV-1900i Plus
Slit Width:	1 nm
Photometric Data:	Absorbance
Photometric Mode	
Measured Wavelengths	665, 750 nm
Integration Time	1.0 sec
Spectral Mode	
Measured Wavelength Range	600 to 800 nm
Scanning Speed	Low

Absorbance was measured at 665 nm and 750 nm (A_{665}, A_{750}). Part of the remaining test solution was also acidified by combining with 0.01 mL of hydrochloric acid (3 mol/L) per 10 mL of test solution, mixing, leaving for 5 to 30 minutes, then measuring absorbance at 665 nm and 750 nm (A_{a665}, A_{a750}). The concentration of chlorophyll a is defined by equation (5) below, where V_e is the volume (mL) of extracted solution, V_s is the volume (L) of test water that was filtered, and d is the light path length (cm) of the absorbance cell.

$$\text{Chlorophyll a (μg/L)} = (\mathrm{A} - A_a) \times 29.6 \times \frac{V_e}{V_s \times d} \qquad (5)$$

Where,

$\mathrm{A} = A_{665} - A_{750}$:
difference in absorbance of test solution before acidification

$A_a = A_{a665} - A_{a750}$:
difference in absorbance of test solution after acidification

As shown in Fig. 2 for acetone extraction, equation (5) was configured in the software. Columns were also added from the factor settings window for entering the volume of extracted solution and filtered test water. A column was also added for entering absorption data that would be used in calculations between rows.

Fig. 5 shows data measured in photometric mode and the calculated results, and Fig. 6 shows example spectra recorded in spectral mode.

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	Sample Name	WL665.0	WL750.0	AorAa	chlorophyll_a	Ve	Vs	d	Aa
1	tap water before acidification1	0.0033	0.0034	−0.0001	−0.0592	20.0000	1.0000	1.0000	0.0000
2	tap water before acidification2	0.0050	0.0047	0.0003	−0.0592	20.0000	1.0000	1.0000	0.0004
3	drinking fountain1 before acidification1	0.0024	0.0025	−0.0001	0.0592	20.0000	1.0000	1.0000	−0.0002
4	drinking fountain1 before acidification2	0.0032	0.0032	0.0000	0.1184	20.0000	1.0000	1.0000	−0.0002
5	tap water after acidification1	0.0030	0.0030	0.0000	0.0000	20.0000	1.0000	1.0000	0.0000
6	tap water after acidification2	0.0061	0.0057	0.0004	0.0000	20.0000	1.0000	1.0000	0.0004
7	drinking fountain1 after acidification1	0.0023	0.0025	−0.0002	−0.0000	20.0000	1.0000	1.0000	−0.0002
8	drinking fountain1 after acidification2	0.0026	0.0028	−0.0002	−0.0000	20.0000	1.0000	1.0000	−0.0002
9	extracted cabbage leaves before acidification1	0.0050	0.0017	0.0033	0.8880	20.0000	1.0000	1.0000	0.0018
10	extracted cabbage leaves before acidification2	0.0110	0.0008	0.0102	2.4864	20.0000	1.0000	1.0000	0.0060
11	extracted cabbage leaves after acidification1	0.0027	0.0009	0.0018	0.0000	20.0000	1.0000	1.0000	0.0018
12	extracted cabbage leaves after acidification2	0.0065	0.0005	0.0060	0.0000	20.0000	1.0000	1.0000	0.0060

Fig. 5 Photometric Results Window: Ethanol Extraction

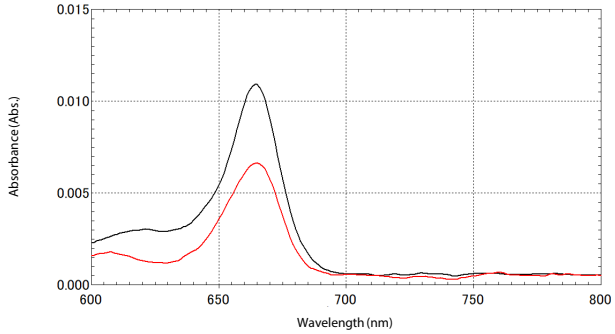

Fig. 6 Ethanol Extraction: Example Chlorophyll Spectra
Solution extracted from cabbage leaf.
Black line: before acidification, red line: after acidification.

Similar to acetone extraction, no chlorophyll a was detected in the municipal water from the building or from the park drinking fountain. The cabbage leaf extract produced an absorbance peak at 665 nm, and the concentration of chlorophyll a in the cabbage leaf sample was calculated from the difference in absorbance before and after acidification. As shown in Fig. 5, in addition to columns for the volume of extracted solution and test water, columns can also be added for entering absorption data of other rows. Although this absorption data must be entered manually, once entered, they can also be used in calculations between rows.

■ Conclusion

This article describes using LabSolutions UV-Vis (Ver. 1.21) in photometric mode and configuring complex equations to calculate the concentration of chlorophyll.

From LabSolutions UV-Vis Ver. 1.21 onwards, quantitation/photometric mode allows the user to add columns that can be populated with figures used in calculations on a row-by-row basis, enabling the user to build equations that incorporate the sample volume and original liquid volume. The ability to build a variety of different equations within a single software promises to reduce human error and streamline the report preparation process. LabSolutions UV-Vis has been designed with ease-of-use as a priority and incorporates features that streamline a variety of tasks.

References

1) Water Standard Test Methods 2020 Edition III Organic Materials, Japan Water Works Association

Related Applications

1. Quantitative Analysis of Chlorophyll a, b and Carotenoids in Cabbage : Use of Evaluation Function of LabSolutions UV-Vis
Application News No. A631

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