Application Note

Environmental

Determination of Chlorophyll-a in Water by UV-Vis Spectroscopy According to APHA 10150B

An efficient workflow using the Agilent Cary 3500 Multicell UV-Vis spectrophotometer



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Abstract

Monitoring chlorophyll-a concentrations provides valuable insight into algal growth, eutrophication, and overall ecosyste m health. This application note demonstrates the determination of chlorophyll-a in environmental water samples using an Agilent Cary 3500 Multicell UV-Vis spectrophotometer in accordance with APHA Standard Method 10150B. Combined with Agilent Cary UV Workstation software, the Cary 3500 Multicell UV-Vis spectrophotometer enables accurate, efficient, and high-throughput chlorophyll-a analysis through simultaneous measurement of multiple samples and automated chlorophyll-a and pheophytin-a calculations.

Introduction

Chlorophyll-a is the primary photosynthetic pigment present in algae. It is widely used as an indicator of phytoplankton biomass, photosynthetic activity, and the trophic state of aquatic environments.[1,2] Monitoring chlorophyll-a concentrations can provide valuable information about algal growth and support the assessment and management of water quality.[1,2]

UV-Vis spectroscopy is a well-established technique for determining chlorophyll-a in environmental water samples. Standardized methods such as APHA Standard Method 10150 and ISO 10260 describe spectrophotometric procedures in which algal cells are collected by filtration, the pigments are extracted into a suitable solvent, and the absorbance measurements are used to quantify chlorophyll concentrations.[1,2] Although the methods differ in sample preparation and extraction procedures (90% acetone, 10% saturated $MgCO_3$ solution for APHA and 90% ethanol for ISO), both can be performed on the Cary 3500 Multicell UV-Vis.[1,2]

Chlorophyll degradation products, particularly pheophytin-a, can interfere with chlorophyll-a determination because they absorb within a similar spectral region.[1] In the APHA 10150B spectrophotometric procedure, absorbance is measured at 664 nm before acidification, and at 665 nm after acidification. Absorbance at 750 nm is subtracted from each measurement to correct for turbidity. The corrected values are then used to calculate the concentrations of chlorophyll-a and pheophytin-a.[1]

APHA 10150B also describes a trichromatic procedure for determining chlorophyll-a, chlorophyll-b, and chlorophyll-c in 90% acetone extracts. In this procedure, absorbance is measured at 664, 647, and 630 nm, with the measurement at 750 nm used to correct for turbidity. The corrected absorbance values are applied to a series of equations to calculate the concentration of each pigment in the extract.[1]

In this application note, chlorophyll-a concentrations in environmental water samples were determined according to APHA 10150B using a Cary 3500 Multicell UV-Vis and Cary UV Workstation software (Figure 1).

Experimental

Sample collection

Water samples were collected in triplicate from two small ponds, referred to as Medicine Pond and Physics Pond, located on the campus of Monash University in Australia. A sample volume of 500 mL was collected from approximately 10 cm below the water surface.

Prior to collection, each bottle was rinsed with sample water and filled with the sample. The bottles were wrapped in aluminum foil to protect the samples from light exposure and transferred immediately to the laboratory. If immediate processing is not possible, samples may be stored at 4 °C in the dark prior to extraction to minimize chlorophyll degradation.[1]



Figure 1. The Agilent Cary 3500 Multicell UV-Vis spectrophotometer and Agilent Cary UV Workstation software. The system allows simultaneous analysis of up to seven samples and supports automated calculation of results with pass/fail evaluation. This reduces manual data processing and help improve analytical efficiency and result consistency.

Sample preparation

Sample preparation followed the extraction procedure described in APHA 10150B. Water samples were filtered through 47 mm glass-fiber filters (Advantec glass-fiber filter, grade GC-50, 47 mm) to concentrate algal biomass. Each filter was transferred to a mortar and pestle and macerated with approximately 2 mL of extraction solvent consisting of 90% ACS-grade acetone (Merck, CAS 67-64-1) and 10% saturated magnesium carbonate ($MgCO_3$) solution. The resulting slurry was transferred to a centrifuge tube and diluted to a final volume of 10 mL using the same extraction solvent.

Samples were stored at 4 °C in the dark for two hours to facilitate pigment extraction. Following extraction, samples were centrifuged at 3000 rpm for 20 minutes and the clarified supernatant was transferred into 10 mm quartz cuvettes for analysis (Figure 2).

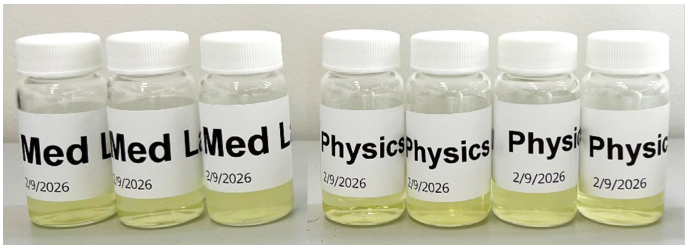


Figure 2. Chlorophyll pigment extracts obtained from Medicine and Physics Pond water samples.

The saturated $MgCO_3$ solution was prepared by suspending 1 g of magnesium carbonate (CAS 546-93-0) in 100 mL Milli-Q water. $MgCO_3$ acts as a buffering agent to minimize chlorophyll degradation during extraction.[1]

Blank solution

A blank solution containing 90% acetone and 10% saturated $MgCO_3$ solution was prepared using the same solvent composition employed for sample extraction. After settling of any undissolved particles, the clarified supernatant was used as the instrument blank.

Instrumentation

Measurements were performed using an Agilent Cary 3500 Multicell UV-Vis spectrophotometer controlled by Agilent Cary UV Workstation software, following the two APHA methods as described as follows.

Trichromatic determination of chlorophyll pigments

The APHA 10150B trichromatic method was used to determine chlorophyll-a, chlorophyll-b, and chlorophyll-c concentrations in acetone extracts. Corrected absorbance values were measured at 664, 647, and 630 nm, with the absorbance at 750 nm used to correct for turbidity. The corrected absorbance values were then used to calculate the concentration of chlorophyll-a, chlorophyll-b, and chlorophyll-c in the extract using the trichromatic equations defined in APHA 10150B. The concentration of chlorophyll-a in the original water sample was subsequently calculated from the pigment concentration in the extract and the sample volume.

Samples were analyzed using a custom Cary UV Workstation method configured according to the APHA 10150B trichromatic procedure (Table 1). Absorbance measurements collected at the required wavelengths were used to automatically calculate chlorophyll concentrations using the APHA trichromatic equations.

Table 1. Data collection parameters for chlorophyll determination using the APHA 10150B trichromatic method.

Parameter	Setting
Wavelength Range	600–800 nm
Analysis Wavelengths	630, 647, 664, and 750 nm
Signal Averaging Time	0.1 s
Spectral Bandwidth	1 nm
Blank	90% acetone, 10% saturated $MgCO_3$ solution
Replicates	3
Path Length	10 mm

Chlorophyll-a and pheophytin-a determination

The APHA 10150B spectrophotometric procedure was used to determine chlorophyll-a in the presence of pheophytin-a, which is a common degradation product of chlorophyll-a. In this method, absorbance measurements were collected at 664 nm before acidification, and at 665 nm after acidification. Measurements at 750 nm before and after acidification were used to correct for turbidity (Table 2). The corrected absorbance values were then used to calculate chlorophyll-a and pheophytin-a concentrations according to the equations specified in APHA 10150B.

Table 2. Data collection parameters for chlorophyll-a and pheophytin-a determination using the APHA 10150B spectrophotometric method.

Parameter	Setting
Wavelength Range	600–800 nm
Analysis Wavelengths	664, 665, and 750 nm
Signal Averaging Time	0.1 s
Spectral Bandwidth	1 nm
Blank	90% acetone, 10% saturated $MgCO_3$ solution
Replicates	3
Path Length	10 mm

Two Cary UV Workstation methods were created. The first method measured absorbance values before acidification. These absorbance values were then transferred as custom parameters to a second method, which measured the acidified extracts and calculated chlorophyll-a and pheophytin-a concentrations according to APHA 10150B.

Results and discussion

Trichromatic determination of chlorophyll pigments

To calculate pigments chlorophyll-a, chlorophyll-b, and chlorophyll-c, the APHA 10150B trichromatic equations were implemented as end-of-sequence calculations in Cary UV Workstation software. The calculations first subtracted the absorbance at 750 nm from the measurements at 664, 647, and 630 nm, then applied the APHA coefficients to calculate C_a, C_b, and C_c in the extract. Sample-specific values, including sample and extract volume, can also be entered as metadata in the sequence table. These parameters were referenced directly by the calculations, allowing samples prepared using different volumes to be processed through the same analytical method without manually modifying the equations. A further calculation incorporated the extract and sample volumes to report chlorophyll-a in the original water sample in mg/m³ (Figure 3).

The trichromatic method showed similar chlorophyll-a concentrations in both ponds, with average chlorophyll-a concentrations of 15.7699 and 14.9287 mg/m³ in the Medicine Pond and Physics Pond, respectively (Figure 4).

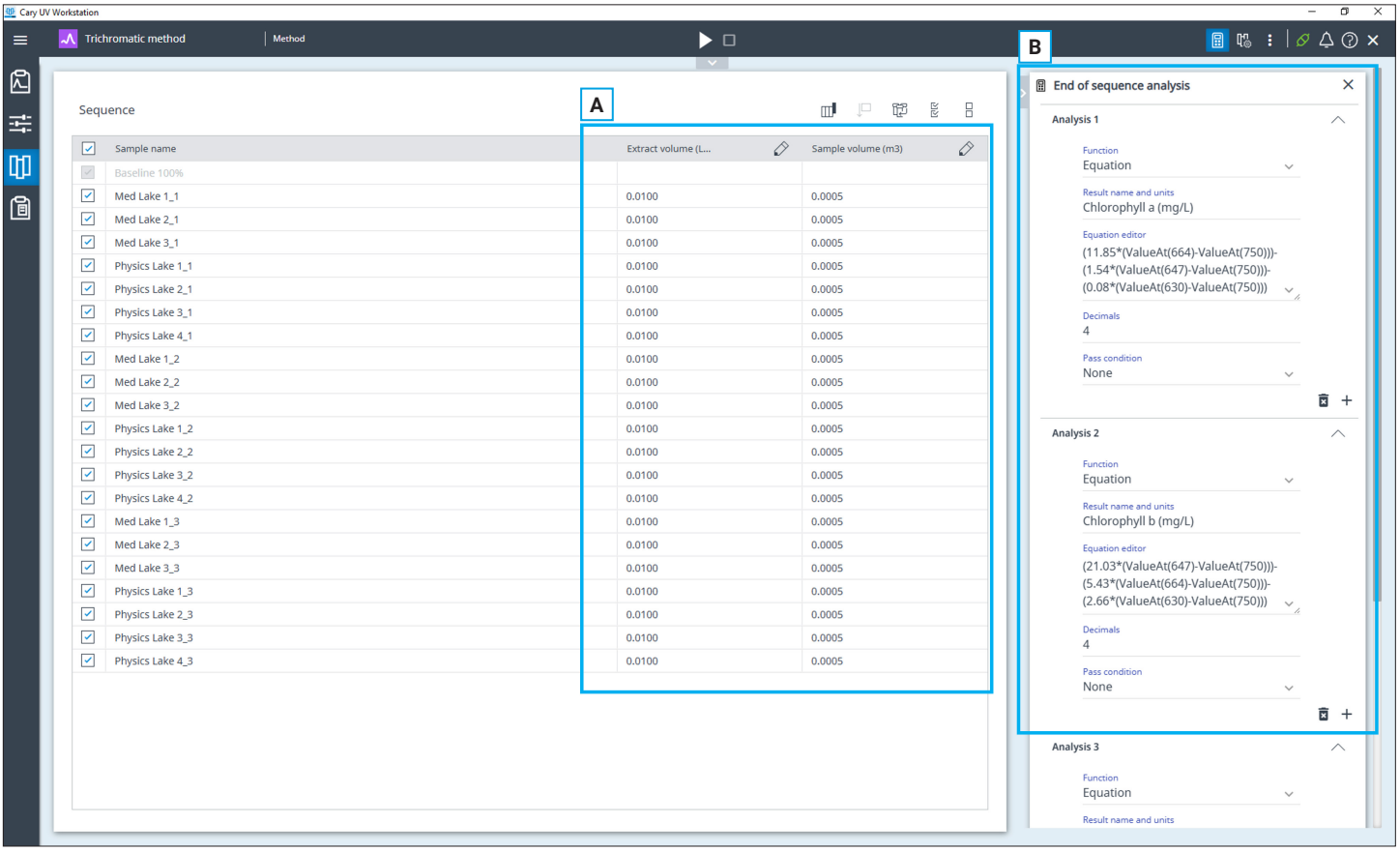
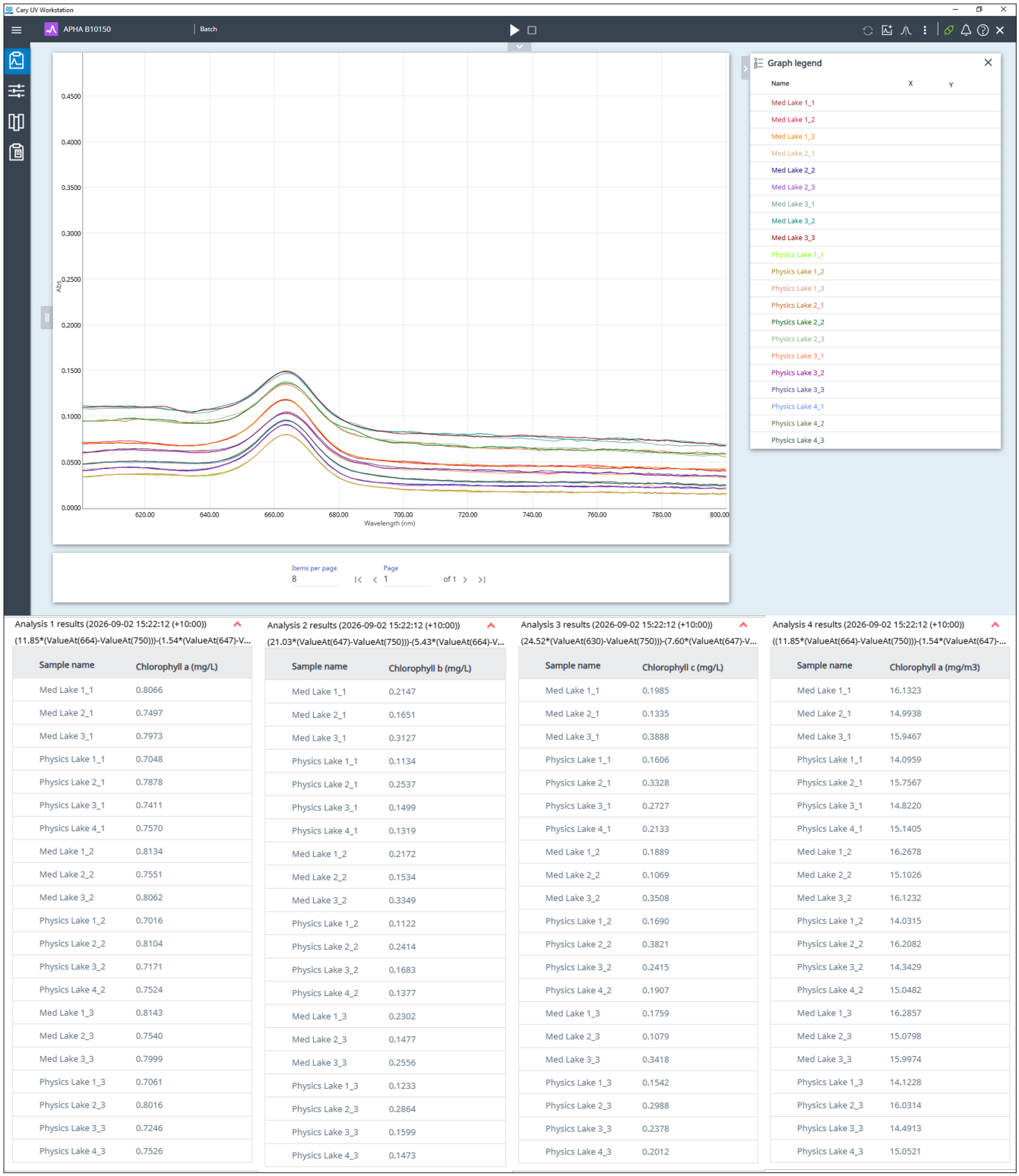


Figure 3. End-of-sequence analysis setup in Agilent Cary UV Workstation software. User-defined parameters, including extract and sample volume (A), are incorporated into custom APHA 10150B equations to automatically calculate chlorophyll-a, chlorophyll-b, and chlorophyll-c concentrations in extracts, and chlorophyll-a concentration in the original water sample (B). Result names, units, and decimal precision are defined within the method for consistent automated reporting.



Analysis 1 results (2026-09-02 15:22:12 (+10:00))
(11.85*(ValueAt(664)-ValueAt(750)))-(1.54*(ValueAt(647)-V...

Sample name	Chlorophyll a (mg/L)
Med Lake 1_1	0.8066
Med Lake 2_1	0.7497
Med Lake 3_1	0.7973
Physics Lake 1_1	0.7048
Physics Lake 2_1	0.7878
Physics Lake 3_1	0.7411
Physics Lake 4_1	0.7570
Med Lake 1_2	0.8134
Med Lake 2_2	0.7551
Med Lake 3_2	0.8062
Physics Lake 1_2	0.7016
Physics Lake 2_2	0.8104
Physics Lake 3_2	0.7171
Physics Lake 4_2	0.7524
Med Lake 1_3	0.8143
Med Lake 2_3	0.7540
Med Lake 3_3	0.7999
Physics Lake 1_3	0.7061
Physics Lake 2_3	0.8016
Physics Lake 3_3	0.7246
Physics Lake 4_3	0.7526

Analysis 2 results (2026-09-02 15:22:12 (+10:00))
(21.03*(ValueAt(647)-ValueAt(750)))-(5.43*(ValueAt(664)-V...

Sample name	Chlorophyll b (mg/L)
Med Lake 1_1	0.2147
Med Lake 2_1	0.1651
Med Lake 3_1	0.3127
Physics Lake 1_1	0.1134
Physics Lake 2_1	0.2537
Physics Lake 3_1	0.1499
Physics Lake 4_1	0.1319
Med Lake 1_2	0.2172
Med Lake 2_2	0.1534
Med Lake 3_2	0.3349
Physics Lake 1_2	0.1122
Physics Lake 2_2	0.2414
Physics Lake 3_2	0.1683
Physics Lake 4_2	0.1377
Med Lake 1_3	0.2302
Med Lake 2_3	0.1477
Med Lake 3_3	0.2556
Physics Lake 1_3	0.1233
Physics Lake 2_3	0.2864
Physics Lake 3_3	0.1599
Physics Lake 4_3	0.1473

Analysis 3 results (2026-09-02 15:22:12 (+10:00))
(24.52*(ValueAt(630)-ValueAt(750)))-(7.60*(ValueAt(647)-V...

Sample name	Chlorophyll c (mg/L)
Med Lake 1_1	0.1985
Med Lake 2_1	0.1335
Med Lake 3_1	0.3888
Physics Lake 1_1	0.1606
Physics Lake 2_1	0.3328
Physics Lake 3_1	0.2727
Physics Lake 4_1	0.2133
Med Lake 1_2	0.1889
Med Lake 2_2	0.1069
Med Lake 3_2	0.3508
Physics Lake 1_2	0.1690
Physics Lake 2_2	0.3821
Physics Lake 3_2	0.2415
Physics Lake 4_2	0.1907
Med Lake 1_3	0.1759
Med Lake 2_3	0.1079
Med Lake 3_3	0.3418
Physics Lake 1_3	0.1542
Physics Lake 2_3	0.2988
Physics Lake 3_3	0.2378
Physics Lake 4_3	0.2012

Analysis 4 results (2026-09-02 15:22:12 (+10:00))
((11.85*(ValueAt(664)-ValueAt(750)))-(1.54*(ValueAt(647)-...

Sample name	Chlorophyll a (mg/m3)
Med Lake 1_1	16.1323
Med Lake 2_1	14.9938
Med Lake 3_1	15.9467
Physics Lake 1_1	14.0959
Physics Lake 2_1	15.7567
Physics Lake 3_1	14.8220
Physics Lake 4_1	15.1405
Med Lake 1_2	16.2678
Med Lake 2_2	15.1026
Med Lake 3_2	16.1232
Physics Lake 1_2	14.0315
Physics Lake 2_2	16.2082
Physics Lake 3_2	14.3429
Physics Lake 4_2	15.0482
Med Lake 1_3	16.2857
Med Lake 2_3	15.0798
Med Lake 3_3	15.9974
Physics Lake 1_3	14.1228
Physics Lake 2_3	16.0314
Physics Lake 3_3	14.4913
Physics Lake 4_3	15.0521

Figure 4. Results table generated automatically in Agilent Cary UV Workstation following analysis using the APHA 10150B trichromatic method. Concentrations of chlorophyll-a, chlorophyll-b, and chlorophyll-c in extracts, and chlorophyll-a in the original water sample, are automatically calculated and reported for each sample.

In terms of the three pigments, chlorophyll-a was the dominant pigment in all samples, with concentrations typically three to four times higher than those of chlorophyll-b and chlorophyll-c. The relatively low variability observed between replicate measurements demonstrates good measurement repeatability and consistent sample processing. The similarity between the two ponds suggests comparable levels of algal biomass at the time of sampling (Table 3).

Implementing the complete calculation workflow within the method allowed the final pigment concentrations to be generated automatically at the end of the sequence. This avoided the need to transfer absorbance values into a separate spreadsheet or calculation package and reduced the potential for transcription, equation-entry, and unit-conversion errors.

Chlorophyll-a and pheophytin-a determination

The APHA 10150B acidification procedure was used to determine chlorophyll-a independently from pheophytin-a, a common chlorophyll degradation product that absorbs within a similar spectral region and can contribute to uncorrected chlorophyll measurements. The method uses turbidity-corrected absorbance values measured at 664 nm before acidification, and at 665 nm after acidification, to calculate chlorophyll-a and pheophytin-a concentrations separately. The seven prepared extracts were analyzed simultaneously using seven measurement positions of the Cary 3500 Multicell UV-Vis, enabling all samples to be processed in parallel before and after acidification. This workflow simplified sample handling and maintained sample order throughout the analysis.

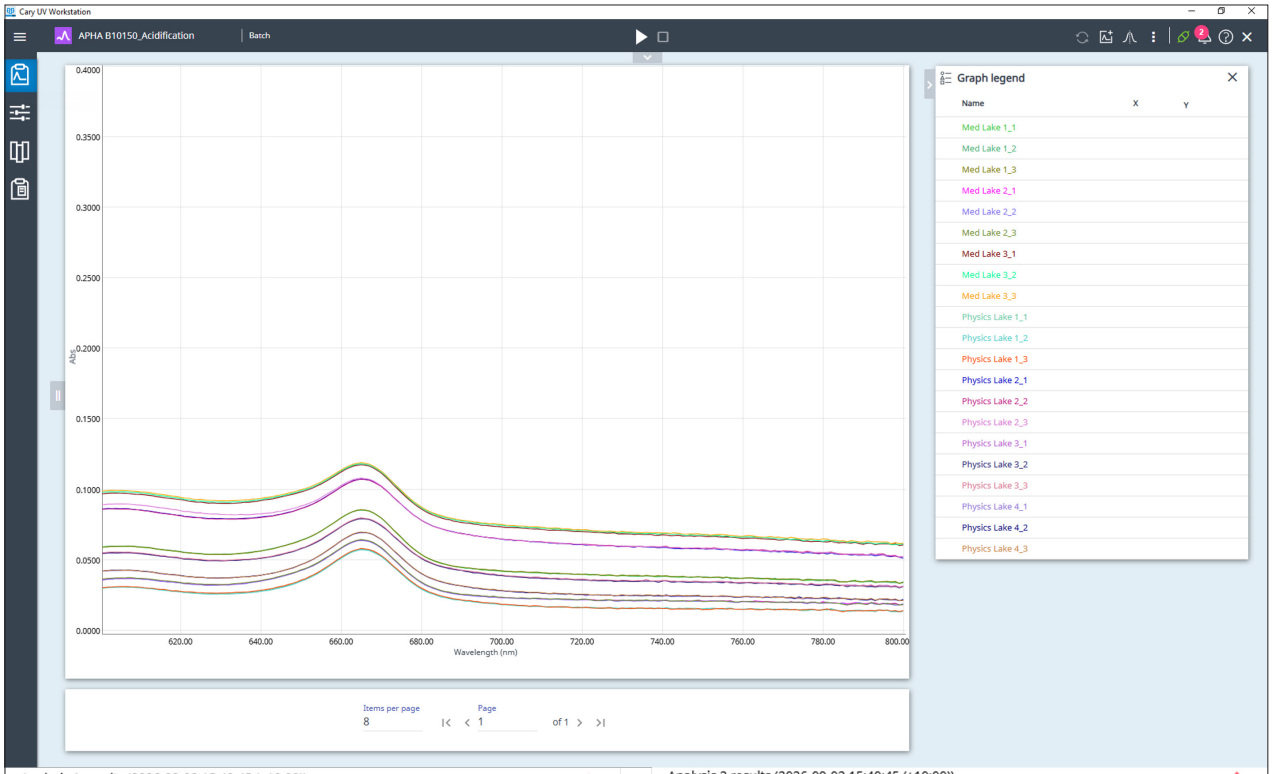
Custom parameters within Cary UV Workstation software were used to store the pre-acidification absorbance values together with the extract and sample volumes for each sample. Following acidification and measurement at 665 and 750 nm, the software automatically applied the APHA 10150B equations to calculate chlorophyll-a and pheophytin-a concentrations at the end of the sequence, eliminating the need for manual calculations and reducing the risk of transcription errors (Figure 5). The resulting chlorophyll-a and pheophytin-a concentrations are summarized in Table 4. Average chlorophyll-a concentrations were 12.7385 and 11.3235 mg/m³ in Medicine Pond and Physics Pond, respectively. The corresponding average pheophytin-a concentrations were 4.9341 and 5.7479 mg/m³, respectively.

Table 3. Chlorophyll-a concentrations in water and chlorophyll pigment concentrations in acetone extracts for samples collected from Medicine Pond and Physics Pond. Values are reported as mean ± standard deviation from three replicate measurements (n = 3).

Sample	Chlorophyll-a in Water (mg/m³)	C_a (mg/L)	C_b (mg/L)	C_c (mg/L)
Medicine Pond 1	16.2286 ± 0.0839	0.8114 ± 0.0042	0.2207 ± 0.0083	0.1878 ± 0.0113
Medicine Pond 2	15.0587 ± 0.0574	0.7529 ± 0.0029	0.1554 ± 0.0089	0.1161 ± 0.0151
Medicine Pond 3	16.0224 ± 0.0909	0.8011 ± 0.0046	0.3011 ± 0.0409	0.3605 ± 0.0249
Physics Pond 1	14.0834 ± 0.0469	0.7042 ± 0.0023	0.1163 ± 0.0061	0.1613 ± 0.0074
Physics Pond 2	15.9988 ± 0.2275	0.7999 ± 0.0114	0.2605 ± 0.0233	0.3379 ± 0.0419
Physics Pond 3	14.5521 ± 0.2453	0.7276 ± 0.0123	0.1594 ± 0.0092	0.2507 ± 0.0192
Physics Pond 4	15.0803 ± 0.0522	0.7540 ± 0.0026	0.1390 ± 0.0078	0.2017 ± 0.0113

Table 4. Chlorophyll-a and pheophytin-a concentrations in water samples collected from Medicine Pond and Physics Pond, determined using the APHA 10150B acidification method. Values are reported as mean ± standard deviation from three replicate measurements (n = 3).

Sample	Chlorophyll-a in Water (mg/m³)	Pheophytin-a in Water (mg/m³)
Medicine Pond 1	13.3804 ± 0.1567	4.4658 ± 0.2663
Medicine Pond 2	12.3939 ± 0.2617	3.8254 ± 0.4449
Medicine Pond 3	12.4412 ± 0.0925	6.5112 ± 0.1573
Physics Pond 1	10.7527 ± 0.4518	5.1577 ± 0.7680
Physics Pond 2	11.8389 ± 0.2475	6.6379 ± 0.4207
Physics Pond 3	10.9809 ± 0.2087	5.8911 ± 0.3549
Physics Pond 4	11.7215 ± 0.0958	5.3049 ± 0.1628



Analysis 1 results (2026-09-02 15:40:45 (+10:00))

26.7*(AbsBeforeAcidification-(ValueAt(665)-ValueAt(750)))*ExtractVolume/(SampleVolume)

Sample name	Chlorophyll a (mg/m3)
Med Lake 1_1	13.4390
Med Lake 2_1	12.5836
Med Lake 3_1	12.4789
Physics Lake 1_1	10.8865
Physics Lake 2_1	11.9366
Physics Lake 3_1	11.2169
Physics Lake 4_1	11.7458
Med Lake 1_2	13.4994
Med Lake 2_2	12.5028
Med Lake 3_2	12.5090
Physics Lake 1_2	11.1224
Physics Lake 2_2	12.0227
Physics Lake 3_2	10.9053
Physics Lake 4_2	11.8028
Med Lake 1_3	13.2029
Med Lake 2_3	12.0954
Med Lake 3_3	12.3358
Physics Lake 1_3	10.2491
Physics Lake 2_3	11.5575
Physics Lake 3_3	10.8205
Physics Lake 4_3	11.6159

Analysis 2 results (2026-09-02 15:40:45 (+10:00))

26.7*((1.7*(ValueAt(665)-ValueAt(750)))-AbsBeforeAcidification)*ExtractVolume/(SampleVolume)

Sample name	Pheophytin a (mg/m3)
Med Lake 1_1	4.3663
Med Lake 2_1	3.5030
Med Lake 3_1	6.4471
Physics Lake 1_1	4.9302
Physics Lake 2_1	6.4718
Physics Lake 3_1	5.4899
Physics Lake 4_1	5.2636
Med Lake 1_2	4.2636
Med Lake 2_2	3.6403
Med Lake 3_2	6.3960
Physics Lake 1_2	4.5292
Physics Lake 2_2	6.3255
Physics Lake 3_2	6.0196
Physics Lake 4_2	5.1667
Med Lake 1_3	4.7676
Med Lake 2_3	4.3330
Med Lake 3_3	6.6904
Physics Lake 1_3	6.0138
Physics Lake 2_3	7.1163
Physics Lake 3_3	6.1638
Physics Lake 4_3	5.4844

Figure 5. Automatically generated results table produced by Agilent Cary UV Workstation software following the APHA 10150B acidification workflow. Chlorophyll-a and pheophytin-a concentrations were calculated automatically from measurements acquired before and after acidification, together with user-defined sample metadata and end-of-sequence calculations.

Measurable pheophytin-a concentrations were observed in all samples, indicating the presence of chlorophyll degradation products. Consequently, chlorophyll-a concentrations obtained using the APHA acidification procedure were lower than those determined using the trichromatic method, reflecting correction for pheophytin-a interference. These results demonstrate the value of the correction step for accurate chlorophyll-a measurements in environmental water samples.

Reporting and pass/fail evaluation

Environmental monitoring laboratories often use internal specifications, regulatory thresholds, or standard operating procedure limits to support routine water quality assessment. Cary UV Workstation software enables these acceptance criteria to be incorporated directly into an analytical method through customizable pass/fail rules. Once defined, calculated chlorophyll results are automatically evaluated against the specified criteria and the outcome recorded as part of the analytical record (Figure 6).

By combining automated calculations, pass/fail evaluation, and reporting within a single workflow, the software helps streamline data review and improve consistency across large sample sets. This capability is particularly valuable in high-throughput environmental laboratories, where rapid result evaluation, traceability, and efficient report generation are essential.

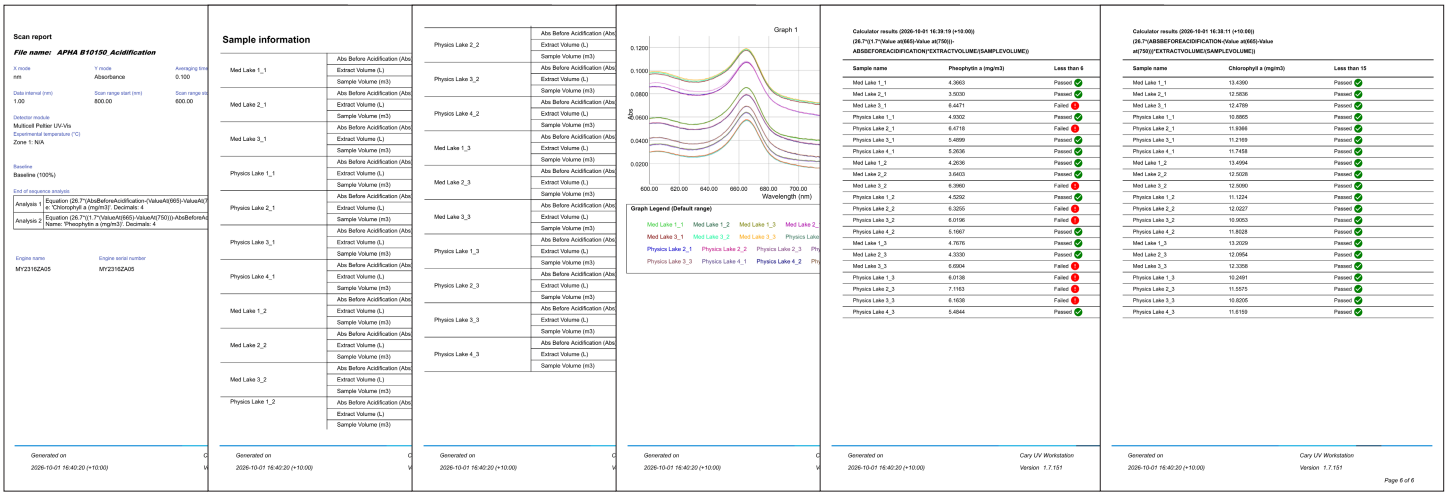


Figure 6. Example report generated by Agilent Cary UV Workstation software showing calculated chlorophyll results and automated pass/fail evaluation. User-defined acceptance criteria can be incorporated directly into the analytical method, allowing results to be assessed automatically and reported immediately following data acquisition.

Conclusion

Chlorophyll-a concentrations in environmental water samples were successfully determined using both the APHA 10150B trichromatic and acidification procedures. Measurable concentrations of pheophytin-a were detected in all samples, demonstrating the presence of chlorophyll degradation products and highlighting the importance of the APHA acidification correction procedure for accurate chlorophyll-a quantification. As expected, chlorophyll-a concentrations determined using the acidification method were lower than those obtained using the trichromatic method due to correction for pheophytin-a interference.

The Agilent Cary 3500 Multicell UV-Vis spectrophotometer and Agilent Cary UV Workstation software provided a streamlined workflow for chlorophyll analysis by combining high-throughput sample measurement with automated implementation of the APHA 10150B calculations. Automated data processing, custom calculations, sample metadata integration, pass/fail evaluation, and report generation reduced manual data handling while enabling rapid delivery of consistent and traceable results. Together, these capabilities improve laboratory productivity and confidence in routine chlorophyll analysis for environmental monitoring applications.

References

1. APHA, AWWA, and WEF. Standard Methods for the Examination of Water and Wastewater, Method 10150: Chlorophyll a, including Section 10150B, Spectrophotometric Determination of Chlorophyll a.
2. International Organization for Standardization. ISO 10260:1992, Water quality: Measurement of biochemical parameters: Spectrometric determination of the chlorophyll-a concentration.

Further information

- Agilent Cary 3500 Multicell UV-Vis Spectrophotometer
- Cary UV Workstation Software
- UV-Vis Spectroscopy FAQs

www.agilent.com/chem/cary-3500-multicell-uv-vis

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