STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF CHLOROPHYLL A AND RELATED PIGMENTS BY HPLC

This document is effective upon final approval

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1.0 Identification of Test Method

1.1 This is the Standard Operating Procedure for the determination of chlorophyll a and pheophytin a in a variety of matrices using high pressure liquid chromatography (HPLC). This revision is effective upon final approval.

1.2 This method is based on EPA Method 447.0.

1.3 Principal modifications of this method from EPA Method 447.0 are the following: the eluent composition, column type, detector, solvents, extraction using sonication and standards and LCS quantification. This method is isocratic composed of methanol and acetone solvents. The stationary phase is a C18 column with an adjoining C18 guard column. A spectrofluorometric detector (excitation 430nm, emission 666nm for chlorophyll a, excitation 409nm, emission 673nm for pheophytin a) is used for quantification and a photodiode array detector is used to record the UV/VIS spectrum of each peak rather than a simple fixed wavelength detector as specified in the reference method. The method is optimized for fast elution of chlorophyll a and pheophytin a. A typical chromatogram is shown in section 24.3 and reference spectra in section 24.4. The chromatographic conditions are shown in section 14.11. Modification also includes use of saturated magnesium carbonate solution (see 10.1 and 14.3.8) to stabilize the pigments in sample extracts.

1.4 This revision reflects incorporation of; isocratic gradient, using only methanol and acetone as solvents, incorporating Instrument Detection Limit (IDL), elimination of the buffer, utilize all-in-one filtration sample vials, establish week long holding time for extract and capture of steep time, changes to duplicate acceptance criterion, linear calibration curves, hold times for standards and LCS changed to six months. Standards and LCS are prepared from known certified stock sources and no longer require quantification. Removal of steeping time needed for standards and increase number of standards required by TNI.

2.0 Applicable Matrices

2.1 This method can be applied to the determination of chlorophyll a and pheophytin a in surface waters and biota samples collected within the South Florida Water Management District.

3.0 Detection Limit

3.1 The detection limit for this method was determined using seven replicate extractions of a typical surface water sample. Analysis was performed on dilutions of the extracts at 3 to 5 times the expected detection limit.

3.2 The method detection limits (MDL's) for this method were determined on 4/17/18 and are shown in the table below. The MDL's are re-verified on an annual basis.

Pigment	MDL(µg/l)	PQL (µg/l)
Chlorophyll a	.024	.1
Pheophytin a	.01	.04

3.3 To achieve the required sensitivity for this method, the procedure includes steps for extraction and concentration of the pigments (see 15.4 for calculations).

3.4 A narrative file, along with the individual values and calculations for the determination of the method detection limits are kept on file with the Laboratory Quality Assurance Officer. The current MDL is reviewed annually by the QA officer and is subject to change. The current MDL is noted on the Test Control Parameters sheet and is available from the QA officer.

3.5 Instrument Detection Limit (IDL) was also calculated by analyzing seven replicates of the low standard. The instrument detection limit was determined to be.

Pigment	IDL(µg/l)
Chlorophyll a	.265
Pheophytin a	.149

4.0 Scope and Application

4.1 This method provides a procedure for determination of chlorophyll a and pheophytin a in surface waters and biota.

4.2 The concentration of chlorophyll is used extensively to estimate phytoplankton biomass, with chlorophyll *a* comprising 1-2% of the algal dry weight. Other pigments that occur in phytoplankton algae are chlorophyll *b* and *c*, xanthophylls, phycobilins and carotenes.

4.3 Chlorophyll a is the dominant type of chlorophyll in the algae most commonly found in surface waters. Pheophytin a is a breakdown product of chlorophyll a and the ratio of chlorophyll to pheophytin a provides information on the health of the algal population. During rapid growth, the proportion of pheophytin a is low. During periods of decline, such as prolonged cloudy weather or exposure of the algae to toxic substances, the proportion of pheophytin a is higher.

5.0 Summary of Test Method

5.1 For surface waters, a well-mixed sample is filtered through a glass fiber filter and pigments are extracted from the retained residue with an aqueous acetone solution using sonication to lyse the algal cells. For biota, the sample is homogenized and freeze dried and a portion of the dried sample is extracted by steeping in an aqueous acetone solution. Concentrations are determined in the extracts using high performance liquid chromatography. An appropriate concentration factor is determined for each sample based on the amount of sample filtered (or mass of dried biota extracted) and the final extract volume. This concentration factor is applied to the concentrations determined in the extract to compute the pigment concentrations in the original sample.

5.2 The working range is up to the concentration of the highest standard for each pigment in the sample extract. If the concentration is greater than the highest standard for a given pigment, a smaller volume can be injected onto the HPLC, or if necessary, the extract can be diluted with the extraction solvent to yield a concentration in the working range. The standard injection volume is 50 uL and can be reduced to a minimum of 10 uL before dilution of the extract is required. The analyst should attempt to select an injection volume/dilution that will yield a concentration near the middle of the working range.

6.0 Definitions

6.1 A comprehensive listing of terms and definitions in common use in the SFWMD laboratory can be found in the Laboratory Quality Manual glossary. All acronyms are defined upon first use in this document. Only terms specific to this procedure are defined in this section.

6.2 An **analytical batch** is a LIMS created grouping of samples and QCs

6.3 **Laboratory Reagent Blank** (LRB) – An aliquot of reagent water that is treated exactly as a sample. The LRB is used to determine if interferences are present in the laboratory environment, reagent, or apparatus.

7.0 Interferences

7.1 Conduct work with chlorophyll extracts in subdued light to avoid degradation. Light and heat cause the chlorophylls and related pigments to degrade and may produce inaccurate results. Use opaque containers or wrap containers with aluminum foil.

8.0 Safety

8.1 Wear safety glasses and a full-length, long-sleeved laboratory coat.

8.2 Use acetone-resistant gloves during filtration, extraction and analysis.

8.3 All personnel conducting this method must be familiar with the SFWMD Chemical Hygiene Plan and must have reviewed Safety Data Sheets for all reagents and standards utilized in this method.

8.3 Avoid contact with skin and eyes while handling samples, sample extracts, and reagents.

8.4 The electrical power should be disconnected before conducting any repairs inside the instrument on controllers, electrical wiring or any other components near sources of electricity.

9.0 Equipment and Supplies

9.1 HPLC System including UV/VIS diode array detector (Shimadzu Prominence), Model LC-2030C 3D and a Spectrofluorometric detector (Shimadzu Prominence), Model RF-20A.

9.2 Chromatography data system (Shimadzu LabSolutions Version 5.86)

9.3 Supelcosil LC-18 15cmX4.6mm, 5um Analytical Column #16731Q-Q1

9.4 Supelguard LC-18 Guard Column Replacement Cartridge 2cm #59564

9.5 Vacuum filtration assembly

9.6 Magnetic filter funnel

9.7 Graduated cylinder, 100-1000 mL capacity

9.8 Pipettes, glass class A

9.9 Eppendorf Reference pipette, 1000 µL (or equivalent)

9.10 Glass Fiber filters - Whatman Type GF/F filter, 47mm, (or equivalent)

9.11 Tweezers or flat–tipped forceps

9.12 Water-proof marker

9.13 Plastic transfer pipettes, disposable

9.14 Amber volumetric flasks, class A, 100 mL

9.15 Cooling Beads (Lab Armor cat#SKU #42370) (or equivalent)

9.16 Sarstedt tubes, item #62.515.006 (or equivalent)

9.17 Silverpak (Ampac Flexibles Fisher Cat#602B-M)

9.18 DPS-20 ultrasonic homogenizer

9.19 Whatman All-in-one Filtration Vial System cat#GN203NPUGMFSP (or equivalent)

9.19 Stir Plate IKA Model: C-MAG MS 4S1 (or equivalent)

9.20 RANIN E4 XLS Electronic Pipette (or equivalent)

9.21 Shimadzu UV-1800 Spectrophotometer

10.0 Reagents and Standards

10.1 **Saturated Magnesium Carbonate Solution** - prepared from Fisher Scientific Cat. No M29-500. Add 1 g finely powdered $MgCO_3$ into a 100 mL class A volumetric flask and dilute to the mark with laboratory reagent water. Shake the solution well and allow it to settle overnight.

10.2 **90% Acetone Solution-** Mix 450mL acetone with 50mL of laboratory reagent water. Shake and mix well.

10.3 **Chlorophyll a Stock Solution (10 mg/L)** – Prepared from chlorophyll a powder, Sigma Cat. No. 1116774-15MG or equivalent with certified concentration. Standards can be made with non-certified sources as well see section 12.8 for procedure. To prepare a 10 mg/L solution, weigh 2 mg (± .1mg) of chlorophyll a powder on a micro analytical balance. Enter the weight on the QC Prep-Standardweight log Transfer the chlorophyll using a glass funnel and glass transfer pipette into a 200mL amber volumetric flask. Dilute to the mark with 90% aqueous acetone solution. Do not freeze. This solution must be prepared every 6 months.

10.4 **Pheophytin a Stock Solution (9.75 mg/L) –** To prepare the pheophytin a stock solution, pipette 25 ml of the chlorophyll a stock solution (10.3) into a 50mL centrifuge tube using a class A volumetric pipette, add 500 µL of Hydrochloric Acid Solution, 0.1005N (Fisher SA54-1) using an Eppendorf pipette and mix well. Do not freeze. This solution must be prepared every 6 months . *9.75mg/L was determined from the theoretical molecular weight ratio (M.W.PheoA /M.W. Chloro A)

10.5 **Acetone (Mobile Phase B)**- HPLC Grade (or equivalent)

10.6 **Methanol (Mobile Phase A)**- HPLC Grade (or equivalent)

10.7 **Laboratory reagent water-** (ASTM Type I water)

10.8 All reagents must be entered online in the Chlorophyll Reagent_Solvent Log with the required information. The server address is kb_wqa\chlorophyll logs.

11.0 Sample Collection, Preservation, Shipment and Storage (Water and Biota)

11.1 Samples are collected using the procedures outlined in the SFWMD Field Sampling Quality Manual, current version.

11.2 Surface water samples that are collected for chlorophyll a and pheophytin a analysis are unfiltered, placed in amber 1 L HDPE bottle and stored at ≤ 6 °C and not frozen.

11.3 Samples are transported to the lab in coolers containing wet ice or other devices to maintain the temperature of the samples at ≤ 6 °C and not frozen until delivery to the SFWMD Laboratory.

11.4 Surface water samples must be filtered within 48 hours from the time of collection. The filters can then be stored in a freezer @ -20°C. Samples must be analyzed within 7 days from the time of extraction, and within 28 days from the date of collection.

11.5 Biota samples should be collected in bags (whirl-paks) which are covered with aluminum foil and stored at ≤ 6 °C until they are delivered to the laboratory. Once the samples are received in the lab, biota samples should be stored at ultra-low temperature (-78+/-2°C) prior to processing.

12.0 Quality Control

12.1 The chlorophyll a and pheophytin a calibration curves and LCS recoveries are checked by the analyst before beginning sample analysis. (See acceptance criterion Section 24.1)

12.2 Standard reference materials are available for chlorophyll a and appropriate control of accuracy is achieved using certified solutions obtained from different sources other than the standards. These control samples are measured after acceptable calibration to verify instrument performance. If the determined values are not within established limits the instrument should be recalibrated with fresh stock standards (see section 13.0) and/or fresh LCS's. If the re-determined value is still not acceptable then the source of the problem must be identified and corrected before continuing analyses. LCS1 and LCS3 are used to confirm instrument calibration and neither are filtered nor sonicated. The % recovery for these

solutions is calculated using the equation shown in section 15.1. Note: Matrix spikes are not feasible for this method and are not used as a quality control measure.

12.2 **LCS1 (Chlorophyll a Control Solution)** – DHI Product ID: PPS-CHLA. The concentration of this commercially obtained standard solution is provided by the vendor. Suitable dilutions are made using 90 % acetone to achieve the approximate target concentration of 1000 **µg/L.** The value calculated from the dilution is entered into LIMS. The vendor supplies 2.5ml of the standard in a serum vial with a septum cap. The actual concentration is reported on the certificate of analysis and is considered the "true value". All vendor/dilution information regarding the stock solution (Lot#, etc.) is recorded in the Chlorophyll QC Preperation_StandararadWeight Log and the Certificate of Analysis is scanned into LIMS standard log using the procedure described in SFWMD-LAB-SOP-5510, current version.

LCS3 (Pheophytin a Control Solution) – DHI Product ID PPS-PHAE. The concentration of this commercially obtained standard solution is provided by the vendor. Suitable dilutions are made using 90 % acetone to achieve the approximate target concentration of 250 **µg/L.** The value calculated from the dilution is entered into LIMS. The vendor supplies 2.5ml of the standard in a serum vial with a septum cap. The actual concentration is reported on the certificate of analysis and is considered the "true value". All vendor/dilution information regarding the stock solution (Lot#, etc.) is recorded in the Chlorophyll QC Preparation_StandardWeight Log and the Certificate of Analysis is scanned into LIMS standard log using the procedure described in SFWMD-LAB-SOP-5510, current version.

12.3 A continuing calibration blank (CCB) is run at the beginning of each run followed by calibration standards and LCS's. Before any samples can be analyzed each must be verified and deemed acceptable. If the CCB is not below the IDL and the LCS concentrations are not within established limits the instrument must be recalibrated. Continuing calibration verification samples (CCV1 (chlorophyll a), CCV2 (pheophytin a) are run every 10 samples and at the end of each run. The % Recovery for CCV1 and CCV2 is calculated using the equation shown in section 15.1.

12.5 **CCV1-1000ug/L Chlorophyll a (Continuing Calibration Verification) –** Standard 5 for chlorophyll a (See table 24.5) is reanalyzed every 10 samples and is also analyzed at the end of the analytical run

12.4 **CCV2 – 200ug/L Pheophytin a (Continuing Calibration Verification) -** Standard 4 for pheophytin a (See table 24.5) is reanalyzed every 10 samples and is also analyzed at the end of the analytical run.

12.5 **Blanks**

Type of Blank QC	Process Contamination Check
LRB	Filtration Equipment
MB	Extraction
CCB	Instrument-baseline

12.5.1 **Laboratory Reagent Blank (LRB)** – Laboratory Reagent Blank (LRB) is prepared every one (1) to twenty (20) environmental samples filtered within twenty four (24) hours. LRB is prepared by filtering 500ml of Laboratory Reagent Water.

12.5.2 **CCB (Continuing Calibration Blank for establishing the "zero point")** – This is a 90% acetone solution (10.2) and is run at the very beginning of each run. If the CCB has a concentration greater than the established IDL the run should be stopped immediately and corrective actions must be taken to resolve the problem (see section 24.1). Under no circumstances should a run where the results of this sample exceed the IDL be accepted.

12.5.3 **Method Blank (MB)-** This is prepared by using a dry clean filter sonicated/extracted just like a sample and then analyzed. MB is prepared along with each batch of samples and is analyzed at the beginning of every run. The blank value must be less than the detection limit for all pigments measured. If the method blank concentration is not below the MDL, the associated sample extracts are suspect of contamination and must be qualified. Only samples that are less than the 10X contamination level require qualifiers.

12.6 **Duplicate (DUP)**-To assess precision associated with sample collection, preservation and storage, as well as laboratory procedures, a duplicate sample should be filtered, extracted and analyzed for every project collected (i.e. each unique P# set). Use a non-blank sample for the duplicate. The duplicate will be labeled aliquot B. The relative percent difference (RPD) of the duplicate set is calculated using the equation shown in section 15.2. The acceptable RPD limit is specified in the Laboratory Quality Manual, current version. If the RPD exceeds the current limit refer to section 24.1 for corrective actions.

12.7 All quality control data must be checked against the currently established limits before entering sample data into the LIMS system. In the event of a QC failure refer to section 24.0 for corrective actions. If the problem(s) cannot be resolved, consult with your supervisor to determine if the samples should be re-run or have appropriate qualifiers added to the result(s). Chronic failure of quality control samples or unusual trends in quality control data should be investigated, documented, and reported to the Laboratory Quality Assurance Officer when they occur.

12.8 Alternate Standard or Quality Controls from uncertified source preparation and quantification

Chlorophyll a Stock Solution (~10 mg/L) – Prepared from chlorophyll a powder, Sigma Cat. No. C5753 or equivalent. To prepare a 10 mg/L solution, weigh ~1 mg of chlorophyll a powder on a micro analytical balance. Record the amount of chlorophyll a in the Chlorophyll Reagents/QC Preparation Log. Transfer the chlorophyll using a glass funnel and glass transfer pipette into a 100mL amber volumetric flask. Dilute to the mark with 90% acetone solution. Completely enclose the volumetric flask in aluminum foil or use amber flasks. Do not freeze. This solution must be prepared every 6 months. To determine the final concentration of the solution, place well mixed sample of the stock solution into a clean 1cm cuvette and measure the absorbance of the solution at 664.3 nm after zeroing the spectrometer with the same cuvette filled with 90% acetone. Record the absorbance and create in-house certificate of analysis (calculation shown below). Record true chlorophyll a concentration in LIMS (see SFWMD-LAB-SOP-5000 (current version)). This solution must be prepared every 6 months.

The concentration of the chlorophyll a stock solution is calculated according to the following equation:

$$[Chlorophyll\ a,\ mg/L] = 1000 \times Abs_{664.3}/87.67$$

Where $Abs_{664.3}$ is the absorbance of the solution at 664.3 nm in a 1cm cuvette, and 87.67 is specific extinction coefficient of chlorophyll a in 90% acetone.

Dilute stock for standards (see section 24.5). For LCS1 the final concentration should be approximately 1000ug/L. (see section 24.5 Standard 5)

Pheophytin a Stock Solution (~10 mg/L) – To prepare the pheophytin a stock solution, pipette 25 ml of the chlorophyll a stock solution (above) into a 50ml centrifuge tube using a class A volumetric pipette, add 500 µL of Hydrochloric Acid Solution, 0.1005N (Fisher SA54-1) using an Eppendorf pipette and mix well. To determine the final concentration of the solution, place a well-mixed sample of the stock solution into a clean 1cm cuvette and measure the absorbance of the solution at 667.0 nm after zeroing the spectrometer with the same cuvette filled with 90% acetone. Record the absorbance and create in-house certificate of analysis (calculation shown below). Record true Pheophytin a concentration in LIMS (see SFWMD-LAB-SOP-5000 (current version)). This solution must be prepared every 6 months.

The concentration of the pheophytin a stock solution is calculated according to the following equation:

$$[Pheophytin\ a,\ mg/L] = 1000 \times Abs_{667.0}/51.20$$

Where $Abs_{667.0}$ is the absorbance of the solution at 667.0 nm in a 1cm cuvette, and 51.20 is specific extinction coefficient of pheophytin a in 90% acetone.

Dilute stock for standards (see section 24.5). For LCS3 the final concentration should be approximately 375ug/L. To prepare place 3.75ml of Pheo A stock solution in 100 ml volumetric flask and fill to line with 90% acetone.

12.9 All reagents, standards and LCS containers must be properly labeled and documented per Laboratory Quality Manual and Chemical Hygiene Plan, current version.

13.0 Calibration and Standardization

13.1 The calibration curve is analyzed in the order of the lowest concentration to the highest concentration and consists of 6 (chlorophyll a) and 5 (pheophytin a) calibration points. The correlation coefficient must be ≥0.995.

13.2 All LCS's must be within established limits.

13.3 Laboratory Blanks must be less than the Method Detection Limit.

13.4 Chlorophyll a and pheophytin a quantitation is based on peak areas.

13.5 All standards must be prepared every 6 months using class A volumetric pipettes and flasks.

13.6 See table 24.5 for standard preparation.

13.7 All LCS's must be prepared every 6 months and are prepared from a separate source or lot number than that of the calibration standards.

14.0 Procedure

Sample Prep-Surface Water Samples

14.1 Create a filtration batch for the analyses to be conducted. Refer to the SFWMD-LAB-SOP-5000 (current version) "Use of Horizon LIMS".

14.2 Chlorophyll samples should be filtered as quickly as possible to minimize the time that samples are exposed to light and room temperature. Light and heat cause the chlorophyll to degrade, which may produce inaccurate results.

14.3 Filtration

14.3.1 The filtration procedure should be conducted in subdued light to avoid degradation, and carried out as quickly as possible to minimize the time that the samples are exposed to light and room temperature.

14.3.2 Referring to the batch, complete a chlorophyll filtration log (Section 24.5 A.) with the following information: the batch number, queue, analyst name, LCS LIMS identifiers and the order in which each sample is processed. The sample ID's can be imported from LIMS. Also record any observations regarding sample composition.

14.3.3 Prepare filtration assembly. All glassware must be clean and acid-free by rinsing with laboratory reagent water and methanol.

14.3.4 Pre-label the Sarstedt tubes with LIMS #, date filtered and project.

14.3.5 Assemble the filter funnel; place a glass fiber filter, rough side up, on top of the filter funnel. The vacuum manifold should be connected to large filter reservoir and a small flask (to keep overflow water out of the vacuum system) via rubber tubing. Turn the vacuum on by opening the valve.

14.3.6 Gently shake the sample container to homogenize the sample. ~500 mL of sample should be filtered. If the sample is high in solids, filter as much sample as can pass through the filter in 10 minutes.

14.3.7 Turn the vacuum on. Pour a measured sample aliquot into the filter funnel and allow to filter completely. If the filter begins to clog, discard and redo filtration using a smaller aliquot. To ensure filtration of entire sample, rinse the graduated cylinder and the funnel with approximately 20 mL of laboratory reagent water and filter. Record the volume of sample filtered in the Filter "A" volume column. The filtration date and time will appear automatically once volume is entered on the log.

14.3.8 After the entire sample has been filtered, dispense 1 mL of $MgCO_3$ solution onto the filter to eliminate degradation of chlorophyll a.

14.3.9 Dry the filter by maintaining the vacuum for an additional 30 seconds, carefully remove the filter, roll filter into a loose tube, roughly the diameter of the centrifuge tube, with algae side inside and transfer into the bottom of the tube. Place the centrifuge tube in a tube rack. Rinse the graduated cylinder and the funnel with approximately 20 ml of 100% methanol and then with 20 ml of laboratory reagent water. Repeat steps 14.3.5- 14.3.7 for the DUP "B" aliquot. Label the two aliquots filtered for each sample "A" and "B".

14.3.10 To send the filtration batch to LIMS see SFWMD-LAB-SOP-5000, current version.

14.3.11 The filters may be stored, frozen (-20°C), for a maximum of 28 days from the date of collection in a foil bag.

14.4 Sample Prep-Biota Samples (periphyton)

14.4.1 Batch periphyton samples for freeze drying procedure (GSLP>FDRY) (Refer to SFWMD-LAB-SOP-1600, current version). Create and print labels for samples that will be prepared.

14.4.2 Place the sample container in a carousel rack filled with cooling beads and homogenize the sample using a Bio Homogenizer equipped with stainless steel tips. Clean the bio homogenizer after every sample.

14.4.3 Sub-sample a portion of the homogenized material and loosely pack a periphyton sample into a labelled Digitube or Black Centrifuge Tube (this step should be conducted under dim light). Cover the Digitube with aluminum foil once the sample has been placed in the container.

14.4.4 Put the Digitubes or Black Centriguge Tubes with the periphyton samples into the freeze dryer and record the starting time (which should be within 48 hours of collection). Refer to SFWMD-LAB-SOP-1600.

14.4.5 When the freeze drying process has been completed, post the FDRY schedules in LIMS (Refer to SFWMD-LAB-SOP-1600, current version) and store the freeze dried samples in a freezer (-20°C). The sample should be extracted and analyzed within 28 days of collection.

14.5 Pigment Extraction (Water and Biota Samples)

14.5.1 Create a batch (extraction) for the analyses to be conducted. The batch should contain a maximum of 40 samples refer to SFWMD-LAB-SOP-5000, current version. Complete a chlorophyll extraction log [Section 24.5 B(water) Section 24.5 C (Biota)] with the following information: the batch number, queue, analyst name, LCS LIMS identifiers and the order in which each sample is extracted. The sample ID's can be imported from LIMS. Also record any observations regarding extraction. Remove the tubes containing the filters or Biota from the freezer. Allow the tubes to thaw for a few minutes in the dark.

14.5.2 For biota samples weigh approximately 50 mg of sample and place in appropriate labelled centrifuge tube. Record sample weight on the extraction log for biota (Section 24.5C).

14.5.3 Dispense 8 mL aqueous acetone solution in each tube using electronic pipette. Gently invert the tubes several times. Uncap tubes and place samples in carousel rack filled with cooling beads. Record the solvent volume used on the extraction log. Also, enter whether the A or B aliquot filter was extracted for water samples. The extraction date and time will appear automatically once volume is entered on log. **Note: Biota samples do not require sonication; proceed with step 14.5.8.**

14.5.4 Sonicator can only hold, per run a total of 18 samples including LCS's. Record the start time and finish time of each sonication run required to complete batch on the Extraction Log. To begin sonication, align probe to the center of the tube (DPS-20 ultrasonic homogenizer manual)

14.5.5 Rinse tubes (2) must be placed in the positions 16 and 17 (they are marked with a double ring around the hole). Samples will be placed in all remaining positions.

14.5.6 Place the loaded carousel rack into DPS-20.

14.5.7 Run all samples using DPS-20 Ultrasonic homogenizer program settings:

Stationary bath
Bath time – 5sec
Amplitude – 50%
Run time – 40 sec
Probe rinsed after each sample, wash changed after each tray.

14.5.8 Place the tubes in a rack and place rack in a cooler to protect from light. Steep extracts for a minimum of 2 hours, but not more than 24 hours at ≤6°C and not frozen. The samples need to be shaken at least once during the steeping phase.

14.5.9 After steeping is complete, filter the extract through a 0.45 µm filter. Record the filtration time on the Extraction log. Copy the last samples' extraction date and time. Paste the date and time as values in appropriate field on Extraction Log. The steep time will automatically populate. Confirm that the steep time is acceptable (2-24 hours). Notify supervisor if steep time requirements are not meet as those samples will require an out of hold qualifier.

14.5.10 To send the extraction batch to LIMS see SFWMD-LAB-SOP-5000, current version.

14.5.11 The extract may be stored at (-20°C), for a maximum of 7 days from the date of .45 µm filtration. Notify supervisor if this time is exceeded. Qualifiers will need to be added.

14.6 Analysis by HPLC

14.6.1 Create a LIMS analytical batch. If needed, edit batch to incorporate all needed LCS, DUP, Blanks and STDS, see SFWMD-LAB-SOP-5000, current version.

14.6.2 Using an Eppendorf and a clean tip for each sample, pipette ~1 mL of each filtered sample extract into an HPLC autosampler vial. Place the vials in the autosampler tray according to the sample table.

14.6.3 Open the template batch file in the HPLC software ("Chlorophyll Template Rack X") in the Batch Editor window. The instrument allows adding multiple batches in que. There are template racks for rack locations 1,2 and 4. Rack 3 is reserved for standards and LCS.

14.6.4 Cut and paste all sample ID's and sample names from the LIMS batch into the HPLC template batch file. Also, make sure the correct method (Chlorophyll3180-007) and reporting file (Chlorophyll Fluorescence) is selected. Enter the appropriate dilution factors based on the volume of sample filtered and final extract volume according to the calculation found in section 15.3. Save the newly created batch file using the Batch number assigned by LIMS. Verify that the injection volume is set to 50 µL for all samples. Dilutions may be entered by adjusting injection volumes.

14.6.5 Load Method Chlorophyll3180-007. Download to instrument turn on pump and oven. Allow instrument to stabilize approxiamently 30 minutes.

14.6.6 Hit the "Queue Batch Run" button in the HPLC software to begin the analysis for a single batch. If several batches have been prepped hit "Batch Queue" and instrument will start analysis on batches in sequence. Reports for each sample will be printed into NuGenesis and parsed by the LIMS so that each set of sample results will appear in the autopost pipe. Once the spectra for the calibration curve and LCS's appear, verify correlation coefficient and LCS values fall within QC limits. If they do not meet the established limits stop run and recalibrate. Enter the pressure and areas of standards and LCS on the Pressure_Area LCS log.

14.6.7 When the batch is complete and run checked, vials can be removed and disposed. The instrument maintains a stable temperature of 4°C and will retard degradation if analytical run needs to be repeated. If the run needs to be reanalyzed start method

again, wait for instrument stabilization, select all samples in batch editor and click "Queue Batch Run" the instruement will prompt to confirm selection.

14.6.8 If the run needs to be reprocessed prior to sending to LIMS, the method file must also be saved. The new method must include the batch id number for future reference and the run is sent by printing data set directly. See SFWMD-LAB-SOP-5000 (current version)

14.7 To post data using the autopost pipe Refer SFWMD-LAB-SOP-5000 (current version). Prior to posting data, all chromatograms should be reviewed to ensure proper peak integration and peak morphology. If a change to the peak integration is required, follow the Laboratory Policy for Manual Peak Integration SFWMD-LAB-SOP-5900, current version. Any changes to the integration should be noted on the analytical run, comments section of Chemistry Checklist (see section 24.6) and/or annotated to the electronic copy of the run stored in NuGenesis. Turn in for approval, the Chemistry Checklist, Batch Browser Report and note any dilutions that were required.

14.8 In accordance with the SFWMD Quality Manual, the analyst is required to keep a maintenance and reagent log on the specified instrument.

14.9 Consult your supervisor before making any major changes, adjustments and/or repairs to the instrumentation.

15.0 Calculations

15.1 Recovery of the LCS is calculated as follows:

$$LCS\%\,\mathrm{Recovery} = \frac{LCS\,\mathrm{Result}}{LCSTrueValue} \times 100$$

15.2 The relative percent difference of the duplicate samples is calculated with the following formula:

$$RPD = \frac{(Value1 - Value2)}{Mean} \times 100$$

15.3 The dilution factor for each sample is calculated as follows:

$$Dilution\ Factor = V(e)/V(s)$$

Where V(e) and V(s) are volume (in mL) of extract and sample respectively.

15.4 The reportable Chlorophyll pigment concentrations and DL's of a given sample are calculated automatically in LIMS after data review using calculation:

$$RPC(\mu g/l) = AR \times DF \times CF$$

$$RDL(\mu g/l) = DL \times DF \times CF$$

Where;
RPC = Reportable Pigment Concentration
RDL = Reportable Detection Limit
AR = Instrument Analytical Result
DF = Instrument required Dilution Factor
CF = Concentration Factor (Extract volume / Initial Sample Volume)

16.0 Method Performance

16.1 This method was validated through comparative analysis and through continuing participation in the QUASIMEME double blind study. Comparative data, results from the blind studies, and data from the initial spiking study are maintained by the Laboratory Quality Assurance Officer and are in his office. Detection limits are shown in section 3.2 and the supporting data are available from the Quality Assurance Officer.

17.0 Pollution Prevention

17.1 Standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents to be disposed.

18.0 Data Assessment and Acceptance Criteria for Quality Control Measures

18.1 After the analytical run is complete, check each quality control value to be sure it falls within acceptable limits per Chemistry Laboratory Quality Manual, current version, otherwise appropriate qualifiers must be added to the result.

18.2 Verify the correctness/acceptability of the results (lab and field QC criteria). Note any discrepancies on the general chemistry checklist and submit for supervisor approval. (See section 24.6)

18.3 The sample holding time is 28 days from the date of collection. Notify the supervisor if any samples are out of the holding time.

18.4 Method blank results must be less than the current MDL. Refer to section 24.1 for corrective action.

18.5 Control solutions are analyzed after calibration to verify system performance. The percent recovery must be within current limits. If control solutions values fall outside the currently established acceptance limits, refer to section 24.1 for corrective action.

18.6 The RPD for the duplicate analysis must be within current limits. If the value is outside the currently established acceptance limits, refer to section 24.1 for corrective action.

19.0 Corrective Actions for Out-of-Control Situations

19.1 See the Table 24.1 for corrective actions. The recommended corrective actions in Table 24.1 are intended as general guidelines to initiate the analytical trouble shooting process. In all cases, if the out-of-control condition persists beyond the second step in troubleshooting, notify your supervisor of the condition and request assistance.

20.0 Contingencies for Handling Out-of-Control or Unacceptable Data

20.1 The LIMS system is designed to review for out of control conditions and will apply the appropriate qualifiers upon data review and supervisor approval.

20.2 It may be necessary to obtain technical service to make repairs to instruments that are producing unacceptable data. See the supervisor for assistance in obtaining this service.

21.0 Waste Management

21.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

21.2 After analysis is complete, sample acetone extracts, instrument waste and expired working standards need to be dumped in a large air tight plastic bottle which will capture and hold the waste for proper disposal.

21.3 Weekly dispose all waste downstairs in approved disposal container and enter waste volumes on the Hazardous Waste Generation Record. Waste is collected and diposed by outside company.

21.4 The samples collected for analysis by this method are not hazardous. They are disposed according to SFWMD-LAB-SOP-5030 (current version).

22.0 Instrument Maintenance

22.1 A maintenance logbook for each piece of instrumentation identified in this procedure shall be maintained by the analyst. The maintenance logbook must include the following items:

1. The name or laboratory designation of the instrument and the associated software;
2. The manufacturer's name, type identification, serial number or other unique identification, and the SFWMD inventory control number;
3. Initial calibration records or other checks to confirm that the instrument complies with the method requirements;
4. The current location of the instrument;
5. Manufacturer's instructions, owner's manuals, or reference to their location;
6. Dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of the next calibration;
7. The maintenance plan as appropriate, and documentation of all maintenance carried out to date, including all routine and non-routine maintenance activities and reference material verifications;
8. Records of any damage, malfunction modification or repair to the instrument;
9. The date received and condition when first placed into service.

23.0 References

23.1 SFWMD-LAB-SOP-5000 (current version) "Use of Horizon LIMS"

23.2 Chemistry Laboratory Quality Manual, current version.

23.3 Field Quality Manual, current version.

23.4 EPA Method 447.0 - Determination of Chlorophylls a and b and Identification of Other Pigments.

23.5 EPA Method 446.0 - In Vitro Determination of Chlorophyll a, b, c1+c2 and Pheopigments.

23.6 SFWMD-LAB-SOP-5510 (current version) "HORIZON Standards Log"

23.7 SFWMD-LAB-SOP-5030 (current version) "Sample Disposal"

23.8 Shimadzu high performance liquid chromatograph manual.

23.9 DPS-20 ultrasonic homogenizer manual.

23.10 DEP-SOP-001/01 FS 1000 General Sampling Procedures(page32), Biological Methods Manual, Florida DEP.

23.11 Phytoplankton pigments in oceanography. Jeffrey & Humphrey (1975)

23.12 Strickland, J. D. H., and T. R. Parsons. 1965. A manual of sea water analysis. Bull. Fisheries Res. Board Can. 125, 2nd ed. 203 p.

23.13 Standard Methods (10200H) for the Examination of Water and Wastewater, 22 nd Edition.

23.14 Simple isocratic HPLC methods for chlorophylls and their degradation products, Mantoura, Barlow and Head

24.0 Tables, Diagrams, Flowcharts and Validation Data

24.1 Corrective actions for out-of control laboratory quality controls.

Lab QC Type	Acceptance Criteria	Recommended Corrective Action
Initial Instrument Blank (CCB) Method Reagent Blank (MB)	<MDL and <IDL	Prepare new blank & restart analysis. If same response is obtained, determine cause of contamination (reagents, calibration standards, environment, equipment failure, etc) & eliminate the source of contamination. Samples that are less than 10X the contamination level must be qualified.
Instrument Calibration Standards	Correlation coefficient ≥0.995	Re-analyze standards. If same response is obtained, trouble shoot the instrument and the standard preparation. If same response is obtained, prepare new standards & re-start analysis.
Laboratory Reagent Blank (LRB)	<MDL	Re-analyze LRB. If same response is obtained, determine cause of contamination & eliminate the source of contamination. Samples that are greater less than 10X the contamination level must be qualified.
Laboratory Control Samples	Accuracy within established limits. See Quality Manual	Re-analyze LCS check standard. If same response is obtained, trouble shoot instrument and re-analyze. If same response is obtained, prepare new LCS & re-start analysis.
Replicate/Duplicate Sample	Precision within established limits. See Quality Manual	Qualify only P# affected.

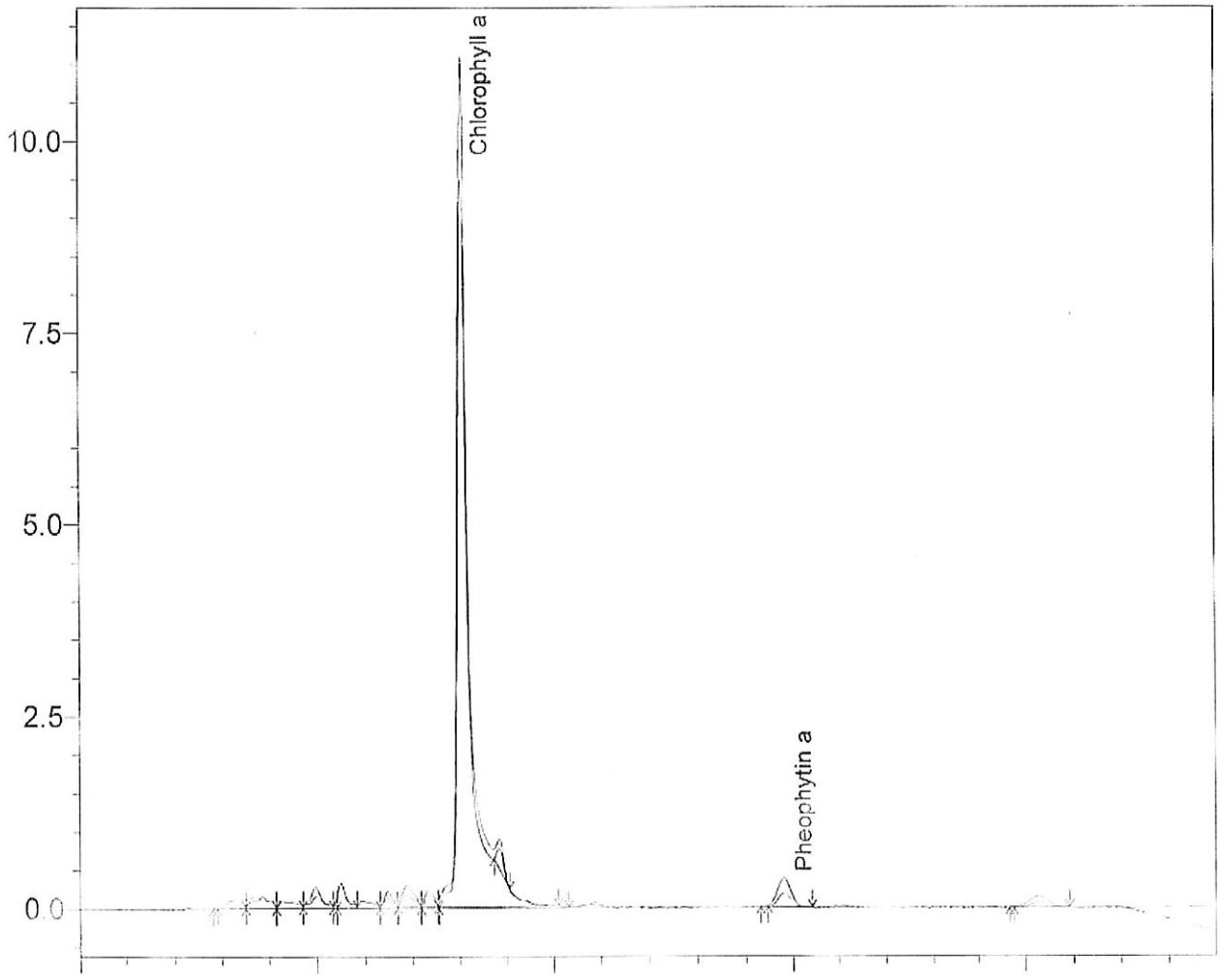
The corrective actions in Table 24.1 are intended as general guidelines to initiate the analytical trouble shooting process. In all cases, if the out-of-control condition persist beyond the second step in troubleshooting, notify your supervisor of the condition and request assistance.

24.2 Corrective actions for out-of-control field quality control samples.

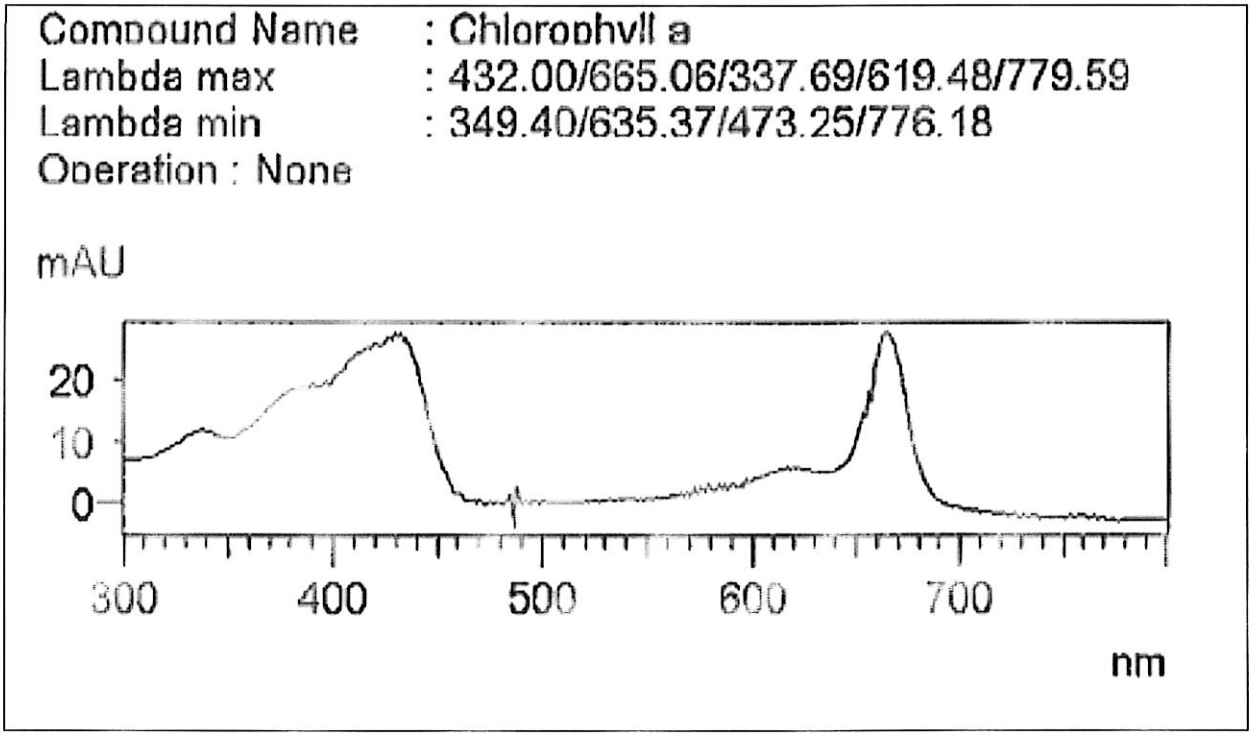
Field QC Type	Acceptance Criteria	Recommended Corrective Action
Equipment Blank(EB) Field Cleaned Equipment Blank(FCEB)	<MDL or less than established limits. See Field Quality manual	Laboratory should re-analyze positive field blanks to confirm results.
Replicate Samples (RS) Field Duplicates (FD)	Precision within limits See Field Quality Manual	Laboratory should re-analyze failing field QC samples to confirm results.

*All repeat analyses must follow procedure as detailed in Section 10.5 of the SFWMD Chemistry Laboratory Quality Manual. Due to limited sample volume and filtering only A and B sample aliquots this analysis cannot be performed a third time for confirmation. Sample data may need qualifiers

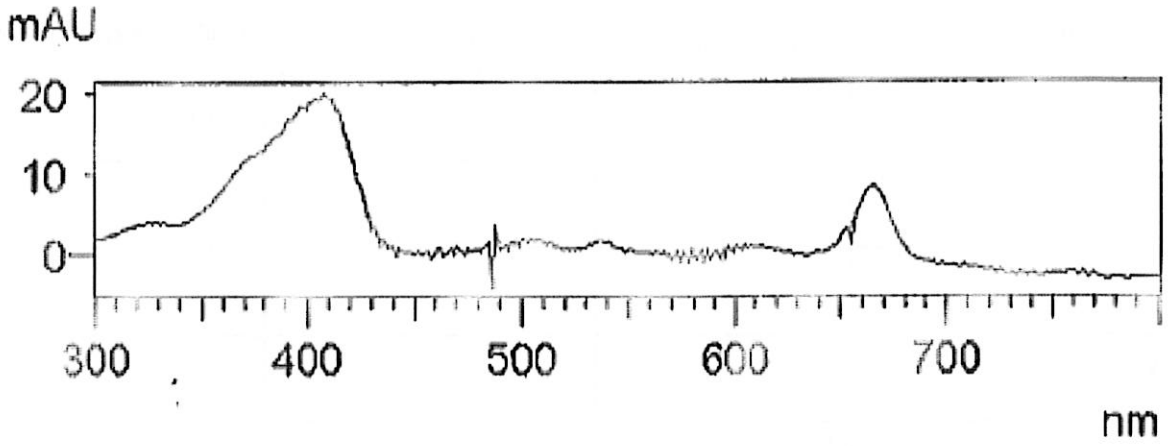
24.3 Typical Chromatogram



24.4 Reference Spectra



Compound Name : Pheophytin a
Lambda max : 407.72/665.67/487.93/329.12/536.95
Lambda min : 338.29/522.02/473.11/458.41/585.29
Operation : None



24.5 Standard Preparation

A. Chlorophyll a Standard Preparation

Standards are made with chlorophyll Solution A (Section 10.3) and 90% Acetone (Section 10.2)

STANDARD	Chlorophyll a solution A (ml)	90% Acetone (ml)	**Final Volume(volumetric)**
Standard 6 (2000ug/L)	20mL	80mL	100mL
Standard 5 (1000ug/L)	10mL	90mL	100mL
Standard 4 (500ug/L)	5mL	95mL	100mL
Standard 3 (200ug/L)	2mL	98mL	100mL
Standard 2 (100ug/L)	1mL	99mL	100mL
Standard 1(5ug/L)	5 mL (Standard 2)	95 mL	100mL
Standard 0 (0ug/L)	0mL	100mL	100mL

B.Pheophytin a Standard Preparation
Standards are made with pheophytin Solution A (Section 10.4) and 90% Acetone (Section 10.2)

STANDARD	Pheophytin a solution A (ml)	90% Acetone (ml)	**Final Volume(volumetric)**
Standard 5 (500ug/L)	5.13mL	95mL	100mL
Standard 4 (300ug/L)	30mL (Standard 5)	20mL	50mL
Standard 3 (200ug/L)	20mL (Standard 5)	30mL	50mL
Standard 2 (100ug/L)	25mL (Standard 3)	25mL	50mL
Standard 1(5ug/L)	5mL (Standard 2)	95mL	100mL
Standard 0 (0ug/L)	0ml	100mL	100mL

24.5 Filtration/Extraction Logs

A. Filtration Log

CHLOROPHYLL FILTRATION LOG (SFWMD LAB SOP XXXX.X)

Queue:	BIOL
Batch:	
Analyst:	

Position	HSN	Filter A Volume (mL)	Filter B Volume (mL)	Filtration Date/Time	Filtration Comments
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					

B. Extraction Log (water)

CHLOROPHYLL EXTRACTION LOG (SFWMD LAB SOP 3180-007)

Queue:	BIOL
Batch:	
Analyst:	

Position	HSN	AVOL (mL)	BVOL (mL)	Filter (A/B)	IVOL (mL)	Extraction Solvent Volume (mL)	Extraction Date/Time	Sonication Begin Time (1)
1								
2								Sonication End Time (1)
3								
4								Sonication Begin Time (2)
5								
6								Sonication End Time (2)
7								
8								Sonication Begin Time (3)
9								
10								Sonication End Time (3)
11								
12								Last Sample Extraction Time (m/d/yyyy h:mm AM or PM)
13								
14								.45um Filtration Time (m/d/yyyy h:mm AM or PM)
15								
16								Steep Time (calculated)
17								
18								
19								Extraction Comments
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
32								
33								
34								
35								
36								
37								
38								
39								
40								
41								
42								
43								
44								
45								
46								

C. Extraction Log (Biota)

CHLOROPHYLL EXTRACTION LOG FOR BIOTA (SFWMD LAB SOP 3180-007)

Queue:	BIOL
Batch:	
Analyst:	

Position	HSN	IWGT (mg)	Extraction Solvent Volume (mL)	Extraction Date/Time	Sonication Begin Time (1)
1					
2					Sonication End Time (1)
3					
4					Sonication Begin Time (2)
5					
6					Sonication End Time (2)
7					
8					Sonication Begin Time (3)
9					
10					Sonication End Time (3)
11					
12					Last Sample Extraction Time (m/d/yyyy h:mm AM or PM)
13					
14					.45um Filtration Time (m/d/yyyy h:mm AM or PM)
15					
16					Steep Time (calculated)
17					0:00:00
18					
19					Extraction Comments
20					
21					
22					
23					
24					

24.6 Chemistry Checklist

CHECKLIST

Analytes / SOP #	CHL-N/SFWMD-LAB-SOP-3180-004
Analysis Date	
ANALYSIS HBN #	
EXTRACTION HBN #	
FILTRATION HBN #	

	ANALYST		SPVR	
Analyst Review	**YES**	**NO**	**YES**	**NO**
Initial Calibration and Quality Control criteria met	X			
Continuing Quality Control and Calibration criteria met	X			
Sample Analysis				
Sample holding times met	X			
Reported Data readings within the calibration range	X			
Samples analyzed out of designated bottle set (if not note in comments)	X			
Other				
Required forms completed and submitted	X			
Above cited SOP read and followed	X			
All reagents and standards documented as required	X			
Electronic Content				
Post Run Annotations		X		
All required documents printed / scanned into LIMS	X			

Comments: _____

Analyst: _____ **Date:** _____
Yelena Potter

Reviewer _____ **Date:** _____
Keith Herring

24.7Method Instrument Parameters

«Header»
Generated : 4/12/2018 10:14:33 AM
GeneratedBy : kher
Modified : 4/12/2018 1:34:29 PM
Modified By : kher

«System Controller»
Model : LC-2030 Controller
EventI : Off
Event2 : Off
Event3 : On
Event4 : On
Degassing Unit : On
Sample Load Timing : Off

<<Data Acquisition»
LC Stop Time : 11.00 min
Detector B Name : Detector B
Detector B Sampling Frequency : 5 Hz
Detector B Start Time : 0.00 min
Detector B End Time : 11.00 min
PDA Detector Name : PDA

«Pump»
Mode : Low pressure gradient
Pump A : LC-2030 Pump
Flow : 1.0000 mL/min
B Conc. : 15.0 %
C Conc. : 0.0 %
D Conc. : 0.0 %
B Curve :0
C Curve :0
D Curve :0
PressMax : 5000 psi
PressMin : 50 psi
LPGE Mode : Auto
Compressibility Setting : Off
Gradient Start Adjustment : None

«Autosampler»
Autosampler Model : LC-2030 Autosampler
Enable Autosampler : Use
Rinsing Volume : 500 uL
Rinsing Speed : 35 uL/sec
Sampling Speed : 5.0 uL/sec
Purge Time : 2.0 min
Rinse Mode : Before aspiration
Rinse Dip Time : 5 sec
On Time Injection : Off
Cooler Temperature : 4 C
Rack Plate L Temperature Control : On
Rack Plate R and Ctrl Rack Temperature Control : On
Air Gap Volume : Off

«Oven»
Oven Model : LC-2030 Oven
Enable Oven : Use
Oven Temperature : 60 C
Maximum Temperature : 90 C

«Detector B»
Model : RF-20A
Lamp On : On
Response : 0.05
Excitation Wavelength : 430 nm
Emission Wavelength : 666 nm
Excitation Wavelength Ch2 : 409 nm
Emission Wavelength Ch2 : 673 nm
Gain : x4
Sensitivity : Medium
Analog Output : Ch1 Integrator
Analog2 Output : Ch1 Recorder

«LC Time Program»

Time	Module	Command	Value
11.00	Controller	Stop	

«Mobile Phase Name»

Mobile Phase A	: Methanol
Mobile Phase B	: Acetone
Mobile Phase C	: Deionized Water
Mobile Phase D	: Deionized Water

«PDA»

PDA Model	: LC-2030/2040 PDA Detector
Lamp	: Off
Start Wavelength	: 280 nm
End Wavelength	: 800 nm
Use Cell Temp.	: Use
Cell Temp.	: 40 C
Slit Width	: 1.2 nm
Polarity	: +
Spectrum Resolution	: 512
Maximum Data Size	: 128MB
Reference Correction	: Not Used
Channe 1 Wavelength	: 666 nm
Channe 1 Bandwidth	: 4 nm
Channe 1 Output Range	: 1.0 AUN
Channe 1 Polarity	: +
Channe 2 Wavelength	: 409 nm
Channe 2 Bandwidth	: 4 nm
Channe 2 Output Range	: 1.0 AUN
Channe 2 Polarity	: +

<<Peak Integration»
<Detector B>

Channel	: Ch1 Ex:430nm,Em:666nm		
Width	: 15 sec		
Slope	: 200 uV/min		
Drift	: 0 uV/min		
T.DBL	: 1000 min		
Max Slices	: 0		
Peak Top Detection	: Normal		
RT Compensation Mode	: Fine		
Min.Area/Height is made effective in Manual Integration	: Off		
Min.Area/Height	: 1000 counts		
Calculated by	: Area		
Noise Calculation Settings	: Noise Data	: Current Data	
	Calculation Method	: ASTM	
	Range	: Whole Range	
	Interval	: 0.5 min	
	Include the Peak Detected Range		: Off
	Detection Limit Coefficient	: 3.3	
	Quantitative Limit Coefficient		: 10.0
Drift Calculation Settings	: 0.000 - 15.000 min		
Auto	: Off		

Channel	: Ch2 Ex:409nm,Em:673nm		
Width	: 10 sec		
Slope	: 200 uV/min		
Drift	: 0 uV/min		
T.DBL	: 1000 min		
Max Slices	: 0		
Peak Top Detection	: Normal		
RT Compensation Mode	: Fine		
Min.Area/Height is made effective in Manual Integration	: Off		
Min.Area/Height	: 1000 counts		
Calculated by	: Area		
Noise Calculation Settings	: Noise Data	: Current Data	
	Calculation Method	: ASTM	
	Range	: Whole Range	
	Interval	: 0.5 min	
	Include the Peak Detected Range		: Off
	Detection Limit Coefficient	: 3.3	
	Quantitative Limit Coefficient		: 10.0
Drift Calculation Settings	: 0.000 - 15.000 min		
Auto	: Off		

«Integration Time Program(Method)»

<Detector B>
Channel : Ch1 Ex:430nm,Em:666nm
Time Program : None

Channel : Ch2 Ex:409nm,Em:673nm
Time Program : None

<<Integration Time Program(Data)>>
<Detector B>
Channel : Ch1 Ex:430nm,Em:666nm
Time Program : None

Channel : Ch2 Ex:409nm,Em:673nm
Time Program : None

<<Identification>>
<Detector B>
Window/Band : Window
Window : 10.00 °A
Identification Method : Absolute
Peak Selection : Largest Peak
Display not identified peaks : Not display
Retention Time Update : Replace

<<Quantitative»
<Detector B>
Quantitative Method : External Standard
Calculated by : Area
# of Calibration Levels : 11
Curve Fit Type : Linear
Zero : Force Through
Weighting Method : None
X Axis of Calib. Curve : Conc.
Units : ug/L
Format of Conc. : Decimals
Format of Conc. Figure : 1
Group Type : Not Used
Check %Dev(Standard) : No
Check Accuracy[%](Standard) : No
Check %Dev(Control) : No
Check Accuracy[%](Control): No
Check %Dev(Additive) : No
Check Accuracy[%](Additive) : No
Check %Dev(Unknown) : No
Check Accuracy[%](Unknown) : No
Check Quantitation Limit : No
Check Detect Limit : No

<<Compound Table»
<Detector B>
ID# : 1
Name : Chlorophyll a
Type : Target
Channel : Ch1 Ex:430nm,Em:666nm
Retention Time : 3.955 min
Retention Index : 0
Concentration : [1]=5 [2]=100 [3]=200
[4]=500 [5]=1000 [6]=2000
Peak Selection : Default(Largest Peak)
Calculated by : Default(Area)
Curve Fit Type : Linear
Zero : Default(Force Through)
Weight : Default(None)
Window/Band : Default(Window)
Spiked : 0.000
1st Coefficient : 1.118158e+003
Intersection : 0.000000e+000
Correction Factor : 1.000000
Standard concentration factor : 1.000000

ID# : 2
Name : Pheophytin a
Type : Target
Channel : Ch2 Ex:409nm,Em:673nm
Retention Time : 7.136 min
Retention Index : 0
Concentration : [7]=5 [8]=100 [9]=200

	[10]=300	[11]=500
Peak Selection	Default(Largest Peak)	
Calculated by	Default(Area)	
Curve Fit Type	Linear	
Zero	Default(Force Through)	
Weight	Default(None)	
Window/Band	Default(Window)	
Spiked	0.000	
Correction Factor	1.000000	
Standard concentration factor		: 1.000000

«Group Table>>
<Detector B>

<<Column Performance»
<Detector B>

Calculation Method	: USP
Unretained Peak Time	: Time at 1st Peak
Column Length	: 150 mm
Calculate Identified Peaks Only	: Off
Calculation of Relative Retention Time	: Off

<<Peak Integration»
<PDA>

Channel	: Ch1 435nm,10nm		
Width	: 2 sec		
Slope	: 200 uV/min		
Drift	: 0 uV/min		
T.DBL	: 1000 min		
Max Slices	:0		
Peak Top Detection	: Normal		
RT Compensation Mode	: Fine		
Min.Area/Height is made effective in Manual Integration	: Off		
Min.Area/Height	: 200 counts		
Calculated by	: Area		
Register Spectrum to Table	: Off		
Noise Calculation Settings	: Noise Data	: Current Data	
	Calculation Method	: ASTM	
	Range	: Whole Range	
	Interval	: 0.5 min	
	Include the Peak Detected Range		: Off
	Detection Limit Coefficient	: 3.3	
	Quantitative Limit Coefficient		: 10.0
Drift Calculation Settings	: 0.000 - 15.000 min		
Auto	: Off		
Channel	: Ch2 665nm,10nm		
Width	: 2 sec		
Slope	: 200 uV/min		
Drift	: 0 uV/min		
T.DBL	: 1000 min		
Max Slices	: 0		
Peak Top Detection	: Normal		
RT Compensation Mode	: Fine		
Min.Area/Height is made effective in Manual Integration	: Off		
Min.Area/Height	: 200 counts		
Calculated by	: Area		
Register Spectrum to Table	: Off		
Noise Calculation Settings	: Noise Data	: Current Data	
	Calculation Method	: ASTM	
	Range	: Whole Range	
	Interval	: 0.5 min	
	Include the Peak Detected Range		: Off
	Detection Limit Coefficient	: 3.3	
	Quantitative Limit Coefficient		: 10.0
Drift Calculation Settings	: 0.000 - 15.000 min		
Auto	: Off		

<<Integration Time Program(Method)>>
<PDA>

Channel	: Ch1 435nm,10nm		
Time Program	: No. Enable	Time(min)	Command
	1 [Yes]	0.000	Integration Off
	2 [Yes]	2.500	Integration On
Channel	: Ch2 665nm,10nm		
Time Program	: No. Enable	Time(min)	Command

	1 [Yes]	0.000	Integration Off
	2 [Yes]	2.500	Integration On

«Integration Time Program(Data)»
<PDA>
Channel : Ch1 435nm,10nm
Time Program : None

Channel : Ch2 665nm,10nm
Time Program : None

«Identification»
<PDA>
Window/Band : Window
Window : 5.00 %
Identification Method : Absolute
Peak Selection : Closest Peak
Display not identified peaks : Not display
Retention Time Update : Replace

<<Quantitative»
<PDA>
Quantitative Method : External Standard
Calculated by : Area
# of Calibration Levels : 11
Curve Fit Type : Linear
Zero : Force Through
Weighting Method : None
X Axis of Calib. Curve : Conc.
Units : ug/L
Format of Conc. : Decimals
Format of Conc. Figure : 1
Group Type : Not Used
Check %Dev(Standard) : No
Check Accuracy[%](Standard) : No
Check %Dev(Control) : No
Check Accuracy[%](Control): No
Check %Dev(Additive) : No
Check Accuracy[%](Additive) : No
Check %Dev(Unknown) : No
Check Accuracy[%](Unknown) : No
Check Quantitation Limit : No
Check Detect Limit : No

<<Compound Table>>
<PDA>

«Group Table»
<PDA>

«Column Performance»
<PDA>
Calculation Method : USP
Unretained Peak Time : Time at 1st Peak
Column Length : 150 mm
Calculate Identified Peaks Only : Off
Calculation of Relative Retention Time : Off

«Multi Chromatogram Table»
Multi Chromatogram Table# :
Ch# 1 : 435 nm, lOnm (x1.00)
Ch# 2 : 665 nm, 10nm (x1.00)
Multi Chrom Mode : Fine
Reference Correction : Not Used
Display Extracted Chromatogram : Off

«UV Spectrum>>
Filtering Type : None
Interpolate Spectra : Off
Background Compensation : Off
Smoothing Points : 0
Lambda min/max Wavelength From : 190 nm
Lambda min/max Wavelength To : 800 nm
Lambda min/max Decimals : 0
Similarity Wavelength From : 190 nm
Similarity Wavelength To : 800 nm
Similarity Decimals : 4

«UV Library Search»

		Index	Parameter
Wavelength From	: 190 nm		
Wavelength To	: 800 nm		
Max # of Hits	: 1		
Prefilter On	: Off		
Prefilter	: Enable		
Use Library in Folder	: No		
Use Library in Specified Folder		: No	
Library File	: 1	C:\LabSolutions\UVLibrary\Phytopigments.11b	

«Purity»

Noise Spectrum 1 Ret. Time From	: 0.2 min
Noise Spectrum 1 Ret. Time To	: 1 min
Compute Noise Spectrum from Current Data	: Off
Wavelength From : 210 nm	
Wavelength To : 800 nm	
Wavelength Step : 1 nm	
Compensation Coefficient : 0	
Background Compensation : On	
Compute Purity Option : Not Calculated	

26.0 Approved Chemical List

CHEMICAL NAME	SOP Section	VENDOR	CATALOG NUMBER
Methanol	14.3.3	Fisher	A454-4
Magnesium Carbonate	10.1	Fisher	M29-500
Hydrochloric Acid Solution	10.5	Fisher	SA54-1
Acetone	10.2 & 10.3	Fisher	A929-04

27.0 Document History

Document Version	Date Issued	Description	Author
001	9/14/10	Original SOP	Y. Guthman
002	9/12/12	New revision of SOP	Y. Guthman
003	6/3/13	New revision of SOP	Y. Guthman
004	6/17/14	New revision of SOP	Y. Potter
005	6/01/16	Removal of chlorophyll b, description of blanks analyzed	Y. Potter
006	5/30/17	New instrumentation update, incorporate filtration logs, general updates	KHerring
007	4/19/18	Method update, isocratic, additional standards, IDL included, Curve additions, LCS and standards hold time increased, solvent changes and general updates	KHerring

26.0 SOP Addendums and Changes