High Performance Liquid Chromatography (HPLC) Method Summary
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Additional information at: http://oceancolor.gsfc.nasa.gov/HPLC/

The current HPLC method has been used since 1999 (Van Heukelem and Thomas 2001, further described in Hooker et al. 2005). This method was used in the SIMBIOS intercalibration exercise (Van Heukelem et al. 2002), and in the SeaWiFS HPLC round-robins: SeaHARRE-1 (Hooker et al. 2000), SeaHARRE-2 (Hooker et al. 2005), SeaHARRE-3 (Hooker et al. 2009), SeaHARRE-4 (Hooker et al. 2010), as well as SeaHARREs 5 and 6 (in preparation).

The HPLC used for pigment analysis is an Agilent RR1200 with a programmable autoinjector (900 µl syringe head), refrigerated autosampler compartment, thermostatted column compartment, quaternary pump with in-line vacuum degasser, and photo-diode array detector with deuterium and tungsten lamps, which collects in-line visible absorbance spectra for each pigment. The HPLC is controlled by Agilent Chemstation software.

The 4.6 x 150 mm HPLC Eclipse XDB column (Agilent Technologies, Palo Alto, CA) is filled with a C8 stationary phase (3.5 µm particle size); the mobile phase consists of a linear gradient from 5% to 95% solvent B over 27 min), for which solvent A is 70 parts methanol, 30 parts 28mM tetrabutylammonium acetate (pH 6.5) and solvent B is methanol. The column temperature is 60ºC and the photo diode array detector is set to plot chromatograms at 450, 665 and 222 nm and to acquire visible absorbance spectra between 350 and 750 nm. The HPLC is fully automated so that analyses can be performed 24 hr per day.

Vitamin E acetate is used as the internal standard (ISTD) for determining extraction volumes. Its absorbance is monitored at 222 nm; it has negligible absorbance at 450 nm and none at 665 nm. Therefore, it does not interfere at wavelengths used to quantify pigments and can be used in very high concentrations with SNR much higher than are possible with pigments. The high SNR contributes to excellent analysis precision, for which injection repeatability averages 0.6%. It is stable under conditions of extraction and analysis and, as an anti-oxidant, may provide benefits to pigment stability.

Calibration is performed with individual pigment standards, whose concentrations have been determined spectrophotometrically using absorption coefficients in common with those used by most other laboratories (Hooker et al. 2005) and the commercial vendor, DHI Water and Environment (Hørsholm, Denmark). Standards are either purchased from DHI (in solution with concentrations provided) or purchased in solid form and suspended in solvent at GSFC (after which the concentrations are determined spectrophotometrically). Thirty-six peaks are individually quantified by HPLC, from which 26 pigments are reported (some pigments contain individual components that are summed and reported as one pigment).

Reported HPLC Pigments

Primary pigments

total chl *a*:	monovinyl + divinyl chlorophyll *a* + allomers and epimers + chlorophyllide *a*
total chl *b*:	monovinyl + divinyl chlorophyll *b*
total chl *c*:	chlorophyll *c*3 + chlorophyll *c*12
carotenes:	carotene pigments (such as alpha and beta), unresolved and therefore undifferentiated
but fuco:	19'-butanoyloxyfucoxanthin
hex fuco:	19'-hexanoyloxyfucoxanthin
diad:	diadinoxanthin
allo:	alloxanthin
diato:	diatoxanthin
zea:	zeaxanthin
fuco:	fucoxanthin
perid:	peridinin + peridinin isomer

Secondary Pigments-pigments that go into calculations of primary pigment products

DV chl *a*:	divinyl chlorophyll *a*
MV chl *a* (or chl *a*):	monovinyl chlorophyll *a* + chlorophyll *a* allomers and epimers
DV chl *b*:	divinyl chlorophyll *b*
MV chl *b* (or chl *b*):	monovinyl chlorophyll *b*
chl *c*3 (or total chl *c*3):	chlorophyll *c*3 + MV chlorophyll *c*3
chl *c*12:	chlorophyll *c*2 + chlorophyll *c*1 + MG DVP (Mg-2,4-divinyl pheoporphyrin a_5 monomethyl ester)
chlide *a*:	chlorophyllide *a*

Tertiary Pigments

lut:	lutein
neo:	neoxanthin
pras:	prasinoxanthin
phide *a*:	a sum of 5 pheophorbide *a* pigments
phytin *a*:	pheophytin *a* + pheophytin *a* prime
viola:	violaxanthin

Ancillary Pigments

gyro diester:	gyroxanthin diester

Reported Sums

DP	total diagnostic pigments (PSC + allo + zea + Tot_Chl_b)
PPC	photoprotective carotenoids (allo + diadino + diato + zea + alpha-beta-car)
PSC	photosynthetic carotenoids (but-fuco + fuco + hex-fuco + perid)
PSP	photosynthetic pigments (PSC + TChl)
TAcc	total accessory pigments (PPC + PSC + Tot_Chl_b + Tot_Chl_c)
TCar	total carotenoids (PPC + PSC)
TChl	total chlorophylls (Tot_Chl_a + Tot_Chl_b + Tot_Chl_c)
TPg	total pigments (TAcc + Tot_Chl_a)

Reported Ratios

PPC_TCar	ratio of photprotective carotenoids to total carotenoids (PPC/TCar)
PPC_TPg	ratio of photoprotective carotenoids to total pigments (PPC/TPg)

PSC_TPg	ratio of photosynthetic carotenoids to total pigments (PSC/TPg)
PSP_TPg	ratio of photosynthetic pigments to total pigments (PSP/TPg)
Tacc_TChla	ratio of total accessory pigments to total chlorophyll a (TAcc/Tot_Chl_a)
TChl_TCar	ratio of total chlorophyll to total carotenoids (TChl/TCar)
TChla_TPg	ratio of total chlorphyll a to total pigments (Tot_Chl_a/TPg)

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