

## SOCOM P06 HPLC

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### **Sample collection**

Near-surface samples from SOCOM CTD stations were taken for HPLC analysis. 1-2 L of sample was filtered in the dark through glass fiber filter (GF/F) having a diameter of 25 mm. Filters were immediately stored in aluminium foil packages in a dewar of liquid nitrogen (-80 °C). Samples were shipped and analyzed at NASA GSFC.

More information on the cruise are available at:

<https://socom.princeton.edu/content/shipboard-data-reports>

### **Analysis method**

The HPLC analysis method can be cited as Van Heukelem and Thomas (2001), further described in Hooker et al. (2005). For a more detailed description, please see below; contact Crystal Thomas (crystal.s.thomas@nasa.gov) for a tailored description.

The HPLC used for pigment analysis is an Agilent RR1200 with a programmable autoinjector (900 ul 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. 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 um stationary phase); the mobile phase consists of a linear gradient from 5-95% solvent B over 27 minutes, for which solvent A is 70 parts methanol, 30 parts 28 mM tetrabutylammonium acetate (pH 6.5) and solvent B is methanol. The column temperature is 60 C and the photodiode array detector is set to plot chromatograms at 450, 665, and 222 nm to acquire visible absorbance spectra between 350 and 750 nm.

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 S:N ratios much higher than are possible with pigments. The high signal:noise ratio contributes to excellent analysis precision, for which injection repeatability averages 0.6%. It is stable under conditions of extraction and analysis.

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 (Horsholm, Denmark). Standards are either purchased from DHI (in solution with concentrations provided) or purchased in solid form and suspended in solvent at GSFC.

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).

Analysis was performed by Lesley Clementson (lesley.clementson@csiro.au) at CSIRO following the protocol of Hooker et al. 2012.

## Abbreviations

### Primary Pigments

Allo	alloxanthin
alpha-beta-Car	carotenes
But-fuco	19'-butanoyloxyfucoxanthin
Diadino	diadinoxanthin
Diato	diatoxanthin
Fuco	fucoxanthin
Hex-fuco	19'-hexanoyloxyfucoxanthin
Perid	Peridinin
Tot_Ch_l_a	total chlorophyll a
Tot_Ch_l_b	total chlorophyll b
Tot_Ch_l_c	total chlorophyll c
Zea	Zeaxanthin

### Secondary Pigments

Chl_c3	Chlorophyll c3
Chlide_a	chlorophyllide a
DV_Ch_l_a	divinyl chlorophyll a
DV_Ch_l_b	divinyl chlorophyll b
MV_Ch_l_a	monovinyl chlorophyll a
MV_Ch_l_b	monovinyl chlorophyll b
	Chlorophyll c2 + chlorophyll c1 + MGDVP
	Mg-2,4-divinyl pheoporphyrin a5 monomethyl ester

### Tertiary Pigments

Lut	Lutein
Neo	Neoxanthin
Phide_a	total pheophorbide a
Phytin_a	total pheophytin a

	Pras	Prasinoxanthin
	Viola	Violaxanthin
<b>Ancillary Pigment</b>	Gyro	Gyroxanthin diester
<b>Other abbreviations</b>		
DP	total diagnostic pigments	PSC + allo + zea + Tot_Ch_l_b
PPC	photoprotective carotenoids	allo + diadino + diato + zea + alpha-beta-car
PPC_TCar	ratio of photoprotective carotenoids to total carotenoids	[PPC]/[TCar]
PPC_TPg	ratio of photoprotective carotenoids to total pigments	[PPC]/[TPg]
PSC	photosynthetic carotenoids	but-fuco + fuco + hex-fuco + perid
PSC_TCar	ratio of photosynthetic carotenoids to total carotenoids	[PSC]/[TCar]
PSP	photosynthetic pigments	PSC + TChl
PSP_TPg	ratio of photosynthetic pigments to total pigments	[PSP]/[TPg]
TAcc	total accessory pigments	PPC + PSC + Tot_Ch_l_b + Tot_Ch_l_c
TAcc_TChla	ratio of total accessory pigments to total chlorophyll a	[TAcc]/[Tchla]
TCar	total carotenoids	PPC + PSC
TChl	total chlorophylls	Tot_Ch_l_a + Tot_Ch_l_b + Tot_Ch_l_c
TChl_TCar	ratio of total chlorophyll to total carotenoids	[TChl]/[TCar]
TChla_TPg	ratio of total chlorophyll a to total pigments	[TChla]/[TPg]
TPg	total pigments	TAcc + Tot_Ch_l_a

## References

Hooker, Stanford B., Lesley Clementson, Crystal S. Thomas, Louise Schlüter, Merete Allerup, Josephine Ras, Herve Claustre, Claire Normandeau, John Cullen, Markus Kienast, Wendy Kozlowski, Maria Vernet, Sumit Chakraborty, Steven Lohrenz, Merritt Tuel, Donald Redalje, Paulo Cartaxana, Carlos R. Mendes, Vanda Brotas, S.G. Prabhu Matondkar, Sushma G. Parab, Aimee Neeley, and Einar Skarstad Egeland. The Fifth SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-5), 2012, NASA/TM-2012-217503.