SOCCOM P18 HPLC

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Sample collection

Near-surface samples from SOCCOM 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 ºC). Samples were shipped and analyzed at NASA GSFC.

More information on the cruise are available at:
https://soccom.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 Crytal 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 tetrabutylammononium acetate (pH 6.5) and solvent B is methanol. The column temperature is 60 C and the photo diode 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).

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_Chl_a	total chlorophyll a
	Tot_Chl_b	total chlorophyll b
	Tot_Chl_c	total chlorophyll c
	Zea	Zeaxanthin
Secondary Pigments	Chl_c3	Chlorophyll c3
	Chlide_a	chlorophyllide a
	DV_Chl_a	divinyl chlorophyll a
	DV_Chl_b	divinyl chlorophyll b
	MV_Chl_a	monovinyl chlorophyll a
	MV_Chl_b	monovinyl chlorophyll b
		Chlorophyll c2 + chlorophyll c1 + MGDVP
		Mg-2,4-divnyl pheoporphyrin a5 monomethyl ester
Tertiary Pigments	Lut	Lutein
	Neo	Neoxanthin
	Phide_a	total pheophorbide a
	Phytin_a	total pheophytin a
	Pras	Prasinoxanthin
	Viola	Violaxanthin
Ancillary Pigment	Gyro	Gyroxanthin diester

Other abbreviations

DP	total diagnostic pigments	PSC + allo + zea + Tot_Chl_b
PPC	photoprotective carotenoids	allo + diadino + diato + zea + alpha-beta-car
PPC_TCar	ratio of photprotective 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 photsynthetic carotenoids to total carotenoids	[PSC]/[TCar]
PSP	phosynthetic pigments	PSC + TChl
PSP_TPg	ratio of photsynthetic pigments to to total pigments	[PSP]/[TPg]

TAcc	total accessory pigments	PPC + PSC + Tot_Chl_b + Tot_Chl_c
TAcc_TChla	ratio of total accessory pigments to total chlorophll a	[Tacc]/[Tchla]
TCar	total carotenoids	PPC + PSC
TChl	total chlorophylls	Tot_Chl_a +Tot_Chl_b +Tot_Chl_c
TChl_TCar	ratio of total chlorophyll to total carotenoids	[TChl]/[TCaro]
TChla_Tpg	raito of total chlorophyll a to total pigments	[TChla]/[TPg]
TPg	total pigments	TAcc + Tot_Chl_a